KARPAGAM ACADEMY OF HIGHER EDUCATION

(Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

(For the candidates admitted from 2018 onwards)

DEPARTMENT OF MICROBIOLOGY

CLASS: I M.Sc (MB) **BATCH – 2018 -2020**

SUBJECT NAME: MARINE MICROBIOLOGY **SUB.CODE**: **I8MBP105A**

SEMESTER: I **4H – 4C**

SYLLABUS

Instruction Hours / week:L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

UNIT – I

Marine microorganisms: collection, preservation, enumeration (total and viable counts), isolation of culture and identification based on morphological, physiological and biochemical characteristics. International and national collection centres.

UNIT – II

Extremophiles: Thermopiles, basophiles, halophiles, psychrophiles, acid – alkalinophiles, oligotroph, toxitolerant, xerotolerant, endolith – Extremophiles and their environment, biodiversity. Genomics of extremophiles, phylogeny of extremophiles, 16S RNA classification in mitochondrial DNA genome, RAPD, RFLP studies.

UNIT – III

Microbiology of degradation of xenobiotic environment: Ecological considerations, decay behaviour, degradative plasmids, hydrocarbons, oil pollution, surfactants, pesticides, Bioremediation:- Factors affecting bioremediation – role of microbes in the marine nutrient cycles – diseases of marine organisms and its impact on marine biodiversity.

UNIT – IV

Brief account of photosynthetic and accessory pigments. Phytoplanktons and Zooplanktons, Red tides, Zones, Bioluminescence and Biopigment, Marine micro and macro organisms, Coral reefs, Mangrooves, Hydrothermal vents and water currents.

UNIT – V

Bar coding of marine organisms: Genome sequencing and physical mapping of genome. Marine exploration, Aquaculture-inland and freshwater, Isolation of marine bioactive compounds-separation, purification and identification techniques, cryopreservation.

SUGGESTED READINGS

TEXT BOOKS

1. Colin Munn. (2011). *Marine Microbiology: Ecology & Applications*. (2nd ed.). Black Well Publishers.
2. Se-Kwon Kim. (2013). *Bioactive compounds and biotechnological applications*. CLS Publishers
3. Joanne, M.W., Linda, S., and Christopher, J.W., (2008). *Prescott, Harley, and Klein's Microbiology*. (7th Ed). McGraw-Hill Higher Education, United States.
4. David Sigee. (2005). *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. (1st ed.). Black well Publishers.

REFERENCES

1. Pelczar, M., JR., Chan, E.C.S., and Noel, R. K., (2006). *Microbiology*. Tata McGraw, Hill. Co. (5th ed.). New Delhi.
2. Dube, H.C. (1994). *A text book of fungi, bacteria and viruses*. Vikas Publishing House, New Delhi.
3. Dale, J.W. (1994). *Molecular genetics of Bacteria*. John Wiley and Stones.
4. Presscott, L.N., Harley, J.P. and Klein, D.A., (1999). *Microbiology*. W.C. Brown Publishers.
5. Stanier, R.Y., Ingharam, J.L., Wheelis, M.L., and Painter, P.R., (1986). *General Waste water engineering Treatment, Disposal and Reuse*. Metcaff and Eddy. Inc., Tata Mc Grew Hill, New Delhi.

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(Deemed to be University Established Under Section 3 of UGC Act, 1956)

Eachanari Post, COIMBATORE - 641 021, INDIA

I-M.Sc., Microbiology (Batch 2018-2020)

Marine Microbiology (Semester-I) (18MBP105A)

LECTURE PLAN

UNIT1

S. no		Lecture duration(Hr)	Topics covered		Supporting materials
1		1	Marine microbes – Collection, Preservation		W1
2		1	Enumeration TVC		T1 99-111
3		1	Isolation and identification of marine microbes		T1 110-121
4		1	Morphological identification		T2 39-79
5		1	Physiological identification		T2 39-79
6		1	International and national collection centres		W1
7		1	Unit I test		

Textbooks : T1-Microbiology- pelczar – McGraw Hill publishing
T2-Microbiology- presscott – McGraw hill publishing
T3-Microbial genetics – David friefelder-

Reference books:

Website: W1- www.microbiology.com
W2 – www.marinestudy.com

Journals:

Prepared by: Mrs. KEERTHANA. K Assistant Professor Department of Microbiology

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LECTURE PLAN

UNIT II

S. no		Lecture duration(Hr)	Topics covered		Supporting materials
1		1	Extremophiles- Thermophiles, Basophiles		T2 643-646
2		1	Halophiles, Psychrophiles		T2 647-648
3		1	Acid – alkalinophiles, oligotroph Toxitolerant, Xerotolerant		T2 648 - 649
4		1	Endolith – extremophile		T2 652
5		2	Biodiversity		T1 543
6		1	Genomics and phylogeny of extremophiles		T2 673-675
7		1	16s r RNA classification and phylogenetic tree		W1
8		1	RAPD and RFLP		T3 640-645

Textbooks : T1-Microbiology- pelczar – McGraw Hill publishing
T2-Microbiology- presscott – McGraw hill publishing
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LECTURE PLAN

UNIT III

S. no		Lecture duration(Hr)	Topics covered		Supporting materials
1		1	Biodegradation of Xenobiotics		
2		1	Degradative plasmids, hydrocarbons		T2 647-652
3		1	Oil pollution and surfactants, Pesticides		T2 653-655
4		2	Bioremediation- Factors affecting Bioremediation		T2 656-657
5		1	Role of microbes in marine		T2 667-682
6		1	Marine nutrient cycles		T2 667-682
7		1	Disease of marine microbes		T2 667-682
8		1	Marine biodiversity impacts		T2 667-682
9		1	Revision of Unit III		T2 667-682

Textbooks : T1-Microbiology- pelczar – McGraw Hill publishing
T2-Microbiology- presscott – McGraw hill publishing
T3-Microbial genetics – David friefelder-

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Marine Microbiology (Semester-I) (18MBP105A)
LECTURE PLAN

UNIT IV

S. no		Lecture duration(Hr)	Topics covered		Supporting materials
1		1	Photosynthetic pigments		T2 167-168
2		1	Accessory pigments		T2 168 - 200
3		1	Phytoplanktons and Zooplanktons		T2 200-210
4		1	Redtides and Zones		T2 200-210
5		1	Bioluminescence and Biopigment		T2 210-220
6		1	Marine micro and macro organisms		T2 210-220
7		2	Coral reefs and Mangrooves		T2 220-240
8		1	Hydrothermal vents and water currents		T2 240-260
9		1	Revision		

Textbooks : T1-Microbiology- pelczar – McGraw Hill publishing
T2-Microbiology- presscott – McGraw hill publishing
T3-Microbial genetics – David friefelder-

Reference books:

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LECTURE PLAN

UNIT V

S. no		Lecture duration(Hr)	Topics covered		Supporting materials
1		1	Bar coding		W1
2		1	Genome sequencing		W1
3		1	Physical mapping		W1
4		1	Marine exploration		T1 643
5		1	Aquaculture inland and freshwater		T1 646
6		1	Cryopreservation		T1 569
7		1	Bio active compounds		T1 618
8		1	Revision of Unit V		
9		1	Unit V test		

Textbooks : T1-Microbiology- pelczar – McGraw Hill publishing
T2-Microbiology- presscott – McGraw hill publishing
T3-Microbial genetics – David friefelder-

Reference books:

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Journals:

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UNIT – I

Syllabus

Marine microorganisms: collection, preservation, enumeration (total and viable counts), isolation of culture and identification based on morphological, physiological and biochemical characteristics. International and national collection centres.

Introduction of marine microbiology

Microbial Life in Marine Environments

More than 70% of the Earth is covered by ocean. When we think of life in the ocean we often think of fishes and whales, life that is visible to the naked eye, but we may be astounded to learn that most life in the ocean is microbial life' new! Microbes account for more than 70% of ocean biomass and constitute a hidden majority of life that flourishes in the sea. What is even more surrisin is that much of this microbial life remains unknown because we cannot culture it in a test tube and it is difficult to observe in nature.

Sampling tools for the marine environment

Introduction

All methods of physical capture are inherently selective. Small fish may pass through large-meshed nets; large fish may out-swim trawls; gill nets will catch fish mainly of a certain size range. Fish may react differently to fishing gear with respect to species, size, biological state, environmental conditions including ambient light and the acoustic noise field, among many other factors.

|This is why organisms are subdivided out of practical necessity, in that the sampling approach and sample size that are appropriate for one group are often inappropriate for another. The disparity in

appropriate techniques for different sizes of groups of organisms has contributed greatly to the paucity of studies on more than one taxonomic grouping at a given locale.

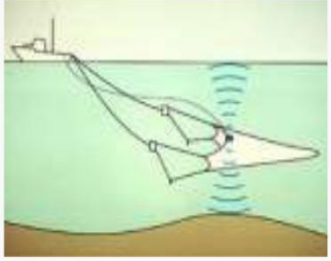
Unfortunately, where conflicting conclusions have been drawn patterns in different groups of organisms, it is rarely possible to know whether the patterns truly vary among groups or merely reflect differences in sampling efforts. The choice of a suitable sampler is a compromise between a variety of factors. Sampling tools for pelagic organisms

Midwater or pelagic trawl

A midwater or pelagic trawl is a set of gear that is used to catch fish that are between the sea surface and bottom, generally staying clear of the bottom. Occasionally, midwater trawls are configured with floats to perform catching in the shallow-surface layer.

A midwater trawl consists of a cone shaped body, normally made of four panels, ending in a codend with lateral wings extending forward from the opening.
Midwater and bottom trawls (see further) have many parts in common, if differing in dimensions and shapes due to their different fishing objects and hydrodynamic regimes of operation. Midwater trawls are designed to catch fish in the midwater column, hence must be capable of rapid maneuvering while maintaining an open net mouth. This is reflected in differences in the body of the net, rigging, and even trawl doors.

pelagic trawl



pelagic trawl

Plankton nets

Plankton nets are a modification on the standard trawl used to collect planktonic organisms, of nearly any size, intact. Towed by a research vessel, plankton nets have a long funnel shape that allows them to catch differently sized plankton simply by changing the mesh size of the net. At the end of the funnel is a collection cylinder called a cod-end.

Ring net

ring net

The ring net consists of a fine-meshed bag attached at its mouth, or opening, to a metallic ring. The net itself is terminated in a bottle or jar where the unfiltered plankton and other particulate matter are collected.

The net is usually deployed vertically for non-quantitative purposes from a platform, such as a vessel or pier. It may also be towed, although lacking in devices for controlling its passage through the water column, which is otherwise determined by hydrodynamic forces generated naturally during towing or hauling. Towing applications are mainly non-quantitative.

Bongo nets

bongo nets

Floating or suspended fish eggs and newly hatched larvae are often caught with Bongo nets. The mesh size is very fine, ranging from 20 µm up to 1000 µm (1 mm), thus allowing eggs and larvae with sizes of order 1-20mm to be caught. The nets, mounted on a rigid yoke, can be towed from the surface to near the bottom for sampling throughout the water column.

In order to obtain quantitative samples of phytoplankton, zooplankton, other invertebrates, and large fish, it is critical to estimate the volume of water that is filtered during the sample. Most bongo and ring nets are deployed with mechanical or electronic flow meters positioned in the mouth of the net to quantify the volume of water filtered.

MOCNESS

(Multiple Opening/Closing Nets and Environmental Sampling System)

MOCNESS

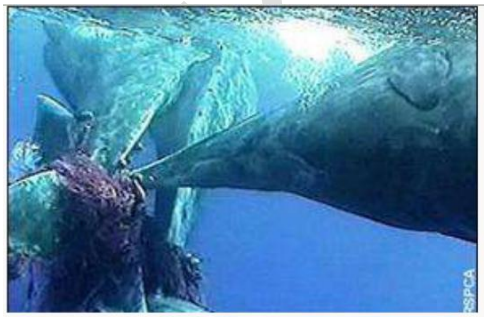
The Multiple Opening/Closing Nets and Environmental Sampling System, which is generally known by its acronym MOCNESS, is an operational, widely used system for capturing plankton at specific depths on the command of the operator. It also routinely carries a number of sensors for measuring environmental parameters as it is towed. These sensors measure, for example, conductivity, temperature, pressure, fluorescence, optical transmission, dissolved oxygen, and light levels.

Neuston nets

neuston net

These types of nets are towed at the surface to sample neuston. Neuston are those organisms associated with the water surface, where they are supported by surface tension. Scientist can determine the number of organisms per unit volume of water filtered.

Drift nets



Whales and dolphins have been caught in drift nets

Drift nets are not set or fixed in any way, are in fact 'mobile', and they are allowed to drift with the prevailing currents. Drift nets are used on the high seas for the capture of a wide range of fish including tuna, squid and shark, and off north-east England for salmon An EU-wide ban on all drift nets was introduced from January 2002 but problems still exist.

Gill nets

gill net

Gill nets are walls of netting which may be set at or below the surface, on the seabed, or at any depth in between. Gill netting is probably the oldest form of net fishing, having been in use for thousands of years. True gill nets catch fish that attempt to swim through the net, which are caught if they are of a size large enough to allow the head to pass through the meshes but not the rest of the body. The fish then becomes entangled by the gills as it attempts to back out of the net. The mesh size used depends upon the species and size range being targeted

Fyke nets

fyke net

Fish communities in shallow water are sampled using fyke nets. A fyke net is a fish trap. It consists of cylindrical or cone-shaped netting bags mounted on rings or other rigid structures. It has wings or leaders which guide the fish towards the entrance of the bags. The fyke nets are fixed on the bottom by anchors, ballast or stakes.

Sampling tools for benthic organisms

The type of gear selected for sampling seabed substrata and the benthic macrofauna at aggregate dredging sites is primarily determined by the hardness/ compactness of the substrata. Whilst a wide variety of sampling methods are available, only a small proportion of these have the ability to effectively collect samples from areas of relatively coarse sediments which are characteristic of dredging sites. In certain situations, it may be necessary to use more than one technique in order to sample the full range of benthic organisms present in an area.

Bottom Grabs

grab

Grab sampling is the simple process of bringing up surface sediments from the seafloor. Once it is launched, the jaws of the grab sampler open and it descends to the seafloor. A spring closes the jaws, and they trap sediments or loose substrate. The grab sampler is then brought up to the surface where its contents are studied in detail.

Hamon grab

Hammon grab

The Hamon grab is the recommended tool for sampling the benthic macro-infauna from coarse substrata. This grab consists of a rectangular frame forming a stable support for a sampling bucket attached to a pivoted arm. On reaching the seabed, tension in the wire is released which activates the grab. Tension in the wire during in hauling then moves the pivoted arm through a rotation of 90°, driving the sample bucket through the sediment. At the end of its movement, the bucket locates onto an inclined rubber-covered steel plate, sealing it completely. This results in the sediment rolling towards the bottom of the sample bucket, thereby reducing the risk of gravel becoming trapped between the leading edge of the bucket and the sample retaining plate, and thus preventing part of the sample being washed out. Weights are attached to the grab to minimize the lateral movement of the supporting frame during sample collection. A drawback of the Hamon grab is that the sediment sample is mixed during the process of collection and retrieval, thereby precluding the examination or sub-sampling of an undisturbed sediment surface.

Van Veen grab

Van Veen grab

The van Veen grab in common with many other grabs, relies on the closure of two opposing jaws for the

collection of a sediment sample. The van Veen grab has long arms attached to each bucket, thus giving better leverage during closure. This mode of action is not ideally suited for the collection of coarse sediments as large particles of gravel tend to become caught between the jaws, resulting in loss of the sample upon retrieval of the grab. Thus, whilst this type of grab has been used widely in benthic macrofauna studies, it is not recommended for use on coarser substrata.

Bottom Trawl

Bottom trawls are commonly used for remotely sampling the epifauna. They are designed to sample at and just above the surface of the seabed and, because of the relatively large area that can be covered in one deployment, they are appropriate for collecting the larger, rarer or more motile species.

The design requirements of a bottom trawl are relatively simple, a mechanism for keeping the mouth of the net open in horizontal and vertical dimensions, a "body" of net which guides fish inwards, and a "cod-

end" of a suitable mesh size, where the fish are collected. The size and design of net used is determined by the species being targeted, the engine power and design of the fishing vessel and locally enforced regulations.

Beam Trawling

The simplest method of bottom trawling, the mouth of the net is held open by a solid metal beam, attached to two "shoes", which are solid metal plates, welded to the ends of the beam, which slide over and disturb the seabed. This method is mainly used on smaller vessels, fishing for flatfish or prawns, relatively close inshore

beam trawl

Otter trawling

Otter trawling derives its name from the "trawl doors" or "otters" which are used to keep the mouth of the net open. As these are towed along the seabed, hydrodynamic pressure pushes them outwards, preventing the mouth of the net closing. They also act like a plough, digging up to 15cm into the seabed, creating a turbid cloud, and scaring fish towards the trawl net mouth. The net is held open vertically on an otter trawl by floats and/or kites attached to the "headline" (the rope which runs along the upper mouth of the net), and weighted "bobbins" attached to the "foot rope" (the rope which runs along the lower mouth of the net). These bobbins vary in their design depending on the roughness of the sea bed which is being fished, varying from small rubber discs for very smooth, sandy ground, to large metal balls, up to 0.5 m in diameter for very rough ground. These bobbins can also be designed to lift the net off the seabed when they hit an obstacle. These trawls are commonly used to collect organisms from a sandy bottom.

otter trawl

Shrimp trawling

shrimp trawl

A special small-mesh bottom trawl is used to catch northern shrimp. This follows the basic design of the otter trawl, but with modified shape and dimensions. The groundrope and sweep are configured to optimize the capture of shrimp.

Dredges

In general, the use of towed dredges for evaluation of epifaunal community structure should be avoided when other sampling tools (e.g. beam trawls) can be effectively employed. However, where the hard or uneven nature of the substrata precludes the use of a trawl it is often possible to obtain adequate samples using dredges, a variety of which are available

Newhaven Scallop dredge

Newhaven Scallop dredge

The Newhaven Scallop dredge is a commercially-used towed device that may be operated over very coarse terrain but would be likely to suffer damage if towed over bedrock or through large boulders. The dredge itself consists of a triangular steel frame supporting, on its underside, a spring-loaded plate to which a tooth bar, designed to dig into the sediment, is bolted. When the dredge encounters rock or large stones, the springs allow the tooth-bar to swing back thus avoiding snagging and reducing the quantity of stones caught. Also attached to each frame is a bag whose lower surface is made up of heavy-duty metal links with an upper surface of heavy gauge nylon mesh. The maximum diameter of particle likely to be retained within the dredge is approximately 20 mm. A number of these dredges may be attached to a robust metal beam which is fitted with large rubber rollers at each end.

The dredges are deployed over the stern or side of a vessel and towed for a pre -determined time. Care must be taken to ensure that the dredge is deployed the right way up. The sampling efficiency of the dredge for each tow can be assessed on deck, normally by the quantity of material collected . The use of this device is recommended for the collection of qualitative samples as a last resort in areas of coarse, unconsolidated sediments which are too rough or uneven to permit the deployment of less robust gear. The Scallop dredge may be used to test the suitability of the ground prior to the deployment of less robust gear (e.g. beam trawl). This may be particularly useful if the ground is thought to be very coarse or uneven.

Rallier-du-bathy dredge

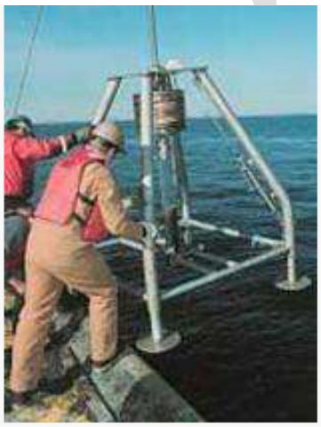
Rallier-du-bathy dredge

The Raillier-du-Baty dredge is designed to work in a range of substrata from sands to cobbles. It consists of a robust metal ring attached to a central towing arm. An open ended bag of the desired mesh size is attached to the ring, and the trailing end of the bag is tied to prevent loss of material during collection of the

sample. This inner bag is protected by an outer, coarser bag which is, in turn, enclosed by a heavy duty apron of fishing net, in order to reduce chafing. The warp is attached to a fixing point on the metal ring, and a weak link is placed between this point and the central arm. This optimizes the digging capability of the edge of the ring and reduces the chances of the edge being lifted away from the seabed. Corers

Corers work by boring a large tube into the benthos and then bringing up a column, or core, of sediment intact within the tube. Caps can automatically seal off the ends of the core after it has pulled up a sample, protecting the sample and keeping it intact. Different sizes and approaches work with different organisms and sediment types.

The gravity corer



gravity corer

Gravity corers are widely used for the collection of the smallest marine metazoans (meiofauna) from subtidal ground. The gravity corer is basically a weighted tube mounted within a frame that descends by

gravity from the research vessel to the sea floor, where it penetrates the sediment to a given depth, filling the tube with sediment in the process. The hydraulically-damped gravity corer has a slow rate of penetration that is controlled by a water-filled piston. Disturbance of the water-sediment interface is minimal and maximum penetration in mud is about 70 cm. A core-catcher on the bottom of the tube moves into place when retrieval begins, trapping the sediment sample in the corer. A winch, onboard the ship, slowly brings the corer back to the surface.

Multiple corer

multiple corer

To test local faunal variations, it is necessary to recover several discrete samples from a single station. The samples could be obtained by multiple casts with a single core tube, but this approach requires much valuable ship time. A multiple corer incorporates the separate coring tubes into a single core body. It consists of a system to which a series of tubes measuring about 4 cm in diameter are attached. Above the system, a weight is mounted and this falls down onto the assembly system when the multicorer touches the sediment. The falling weight drives the tubes into the sea bed so that when they are raised again each of

them contains a drilling core with sediment from the sea floor. When studying the benthic communities, scientists then take account of the depth at which these tiny creatures were found below the sea floor.

Reineck boxcorer

Reineck boxcorer

The boxcorer takes relatively undisturbed samples. The equipment operates by a self releasing trigger system triggered by the frame touching the sea bed. The square box is pushed into the bottom by gravity force of the weight mounted on the top of the box retainer. A spade freed by the trigger-mechanism closes the sample box during the recovery of the unit preventing the sample being washed-out.

Multi boxcorer

multi boxcorer

A multi boxcorer incorporates the separate coring tubes of a boxcorer into a single core body.

Hyperbenthos sledge

The benthic carriage drags a net over the sea floor. This net is divided into various compartments one above the other so that the benthic communities can be collected whether they are close to or further away from the sea floor.

hyperbenthos sledge

Hyperbenthos net

Instruments and sensors to measure environmental parameters Measurement of environmental parameters

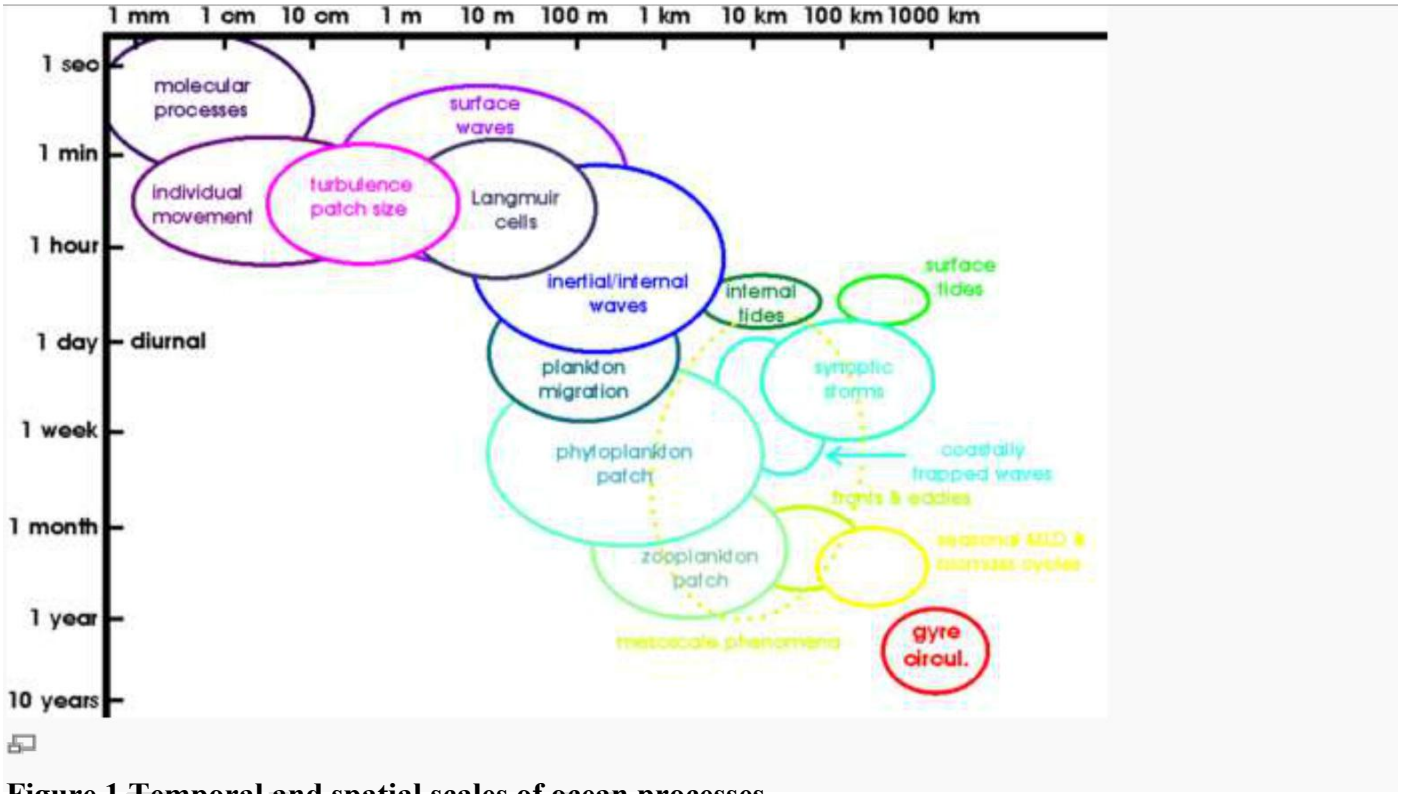


Figure 1 Temporal and spatial scales of ocean processes

The simplest way in which on can measure the environmental parameters of water, is to take samples and then analyze them after returning to the laboratory. It is a powerful approach since specialized laboratory equipment can be used to analyze a multitude of parameters. The main shortcomings of this approach are

that only a limited number of measurements (samples) can be processed and the time between samples taken at the same location (to gain information about the temporal variation) usually

spans from weeks to months. Processes that occur on time-scales shorter than weeks or episodic and transient events are therefore not captured. As a result, the importance of these processes and events for the distribution of parameters cannot be assessed.

In oceanography, there is a vast range of processes spanning many orders of time and space (see Figure 1). To allow for the investigation of these processes, a large volume of data must be gathered on the appropriate
time and space scales. To achieve this task, instruments are needed that measure environmental parameters automatically in situ.

Oceanographic instruments

Introduction

An oceanographic instrument generally consists of one or more sensors as well as a signal processing unit that converts the sensor signal and carries out scaling and conversion to engineering units and to the output data protocol. Figure 2 shows a schematization of an oceanographic instrument. The analyte (property to be measured) interacts with the detector (in some cases after a stimulus has been exerted by the instrument). The detector produces a signal, that is transformed into an electrical signal by the transducer. Detector and transducer together constitute the sensor. The electrical signal is fed to the signal processing (and conditioning) unit that creates the signal output of the instrument.

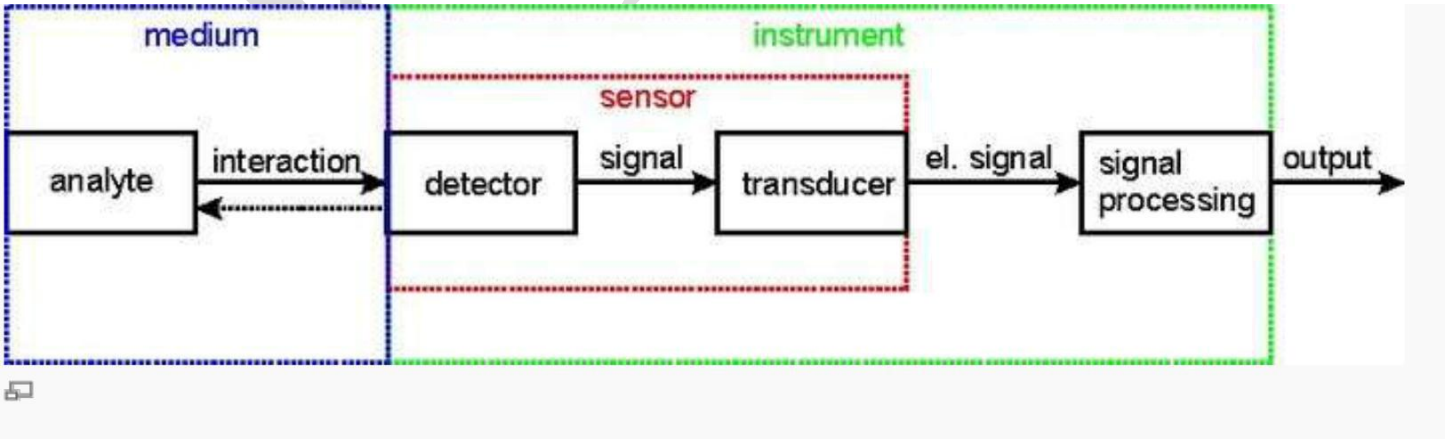


Figure 2 Schematization of a generalised oceanographic instrument

Oceanographic instruments can contain data loggers to store measurement data for readout after the deployment.

Important properties

- **Accuracy**: deviation of the measured value from the true value
- **Precision**: deviation of a measured value from another measured value of the same quantity (but at different environmental conditions (e.g. the two measurements taken at different temperatures)) **Resolution**: smallest change in the measured quantity that can be detected by the instrument
- **Measurement rate**: number of measurements that can be carried out per unit time (e.g. measurements/hour)

- **Power consumption**: mean of electrical power uptake during deployment (usually measured in Watts [W])
- **Deployment time**: time period for which the instrument can be deployed (usually depends on environmental conditions, such as biofouling, or on stored energy and power consumption)

Sensors

Introduction

In an oceanographic instrument the stimulus can interact either directly with the detector (e.g. in a temperature, pressure or light sensor) or a stimulus can be exerted by the instrument. The stimulus is then modified by the property to be measured and then interacts with the detector, such as a fluorometer that sends out a light pulse (stimulus), which is transformed by chlorophyll fluorescence in the water (modification of stimulus). The transformed light (modified stimulus) then interacts with the detector.

If the detector signal is of a property (such as color) it can be converted to an electrical signal by a not an electrical signal (e.g. an optical signal or the change transducer). The sensor is made up of both the detector and the transducer.

Types of sensors

There are numerous sensors in oceanographic work:

Some of the most commonly used are

- Temperature sensors (under construction)
- Salinity sensors (under construction)
- Turbidity sensors such as

Secchi disk

Optical backscatter point sensor (OBS)

Optical transmissiometers (Theme 9 wanted page)

Oxygen sensors

Fluorescence sensors

Multi-probe sensors (Theme 9 wanted page)

Less common are

- pH sensors
- Optical Laser diffraction instruments (LISST)
- Flow cytometers
- pCO2 sensors
- Acoustic point sensors (ASTM, UHCM, ADV)
- Acoustic backscatter profiling sensors (ABS)

Examples of specialized sensor systems are

- Nutrient analyzers
- Trace metal analyzers (Theme 9 wanted page)
- Measuring instruments for fluid velocity, pressure and wave height
- Measuring instruments for sediment transport
- Instruments for bed level detection
- Waverider buoys (under construction)
- Underwater video systems

Important properties

- **Sensitivity**: The smallest change in the property being measured that leads to a measurable change in the detector signal.
- **Selectivity**: How those properties, other than the one being measured, lead to changes in the detector signal. High selectivity sensors exhibit little change in the detector signal from properties other than the one being measured.
- **Range**: The span between the extremes of the property being measured, at which no further change in the detector signal occurs.
- **Linearity**: A measure of how far equal amounts of change in the property being measured, lead to equal amounts of change in the detector signal.

THE AQUATIC ENVIRONMENT:

The microbial population in a body of natural water is, to a large extent, determined by physical and chemical conditions which prevail in that habitat. Some of these conditions are,

Temperature:

The temperature of surface waters varies from near 0 degree celsius in polar regions to 30-40 degree celsius in equitorial regions. More than 90 per cent of marine environment is below 5 degree celsius, a condition favourable for the growth of psychrophilic microorganisms. Microorganisms do occur in natural ho springs where temperature as high as 75-80 degree celsius prevail (*Thermus aquaticus*, a common bacterial

inhabitantof hot springs, has an optimum growth temperature of 70-72 degree celsius). Recently, microbiologist have reported extreme thermophilicmicroorganisms associated with geothermal ventsin the pacific ocean floor. These unusual microbes are said to be capable of growing at 250 degree cesius and 265 atm of pressure.

Hydrostatic Pressure:

There are striking differences in the hydrostatic pressure of surface waters an in waters of oceanic depths. Hydrostatic pressure affects the chemical equilibrium,which, in turn, results in lowering of pH of seawater, resulting in the change in the solubility of nutrients such as bicarbonate. Hydrostatic pressure also increases the boiling point of water, thereby maintainingwater in its liquid state at high temperature and pressures. By definition hydrostatic pressure increases with depth at the rate of 1atm per 10m. Barophilic microorganisms, organisms which cannot grow at normal atmospheric pressures, have been isolated from pacific trenches (depth

1000 to 10,000 m), where enormous hydrostatic pressures exist (greater than 100 atm). Almost all Barophilic bacteria must be grown under psychrophilic conditions (about 2 degree celsius).

Light:

The metabolic products of photosynthetic organisms. In most aquatic habitats these primary producers are algae, and their growth is restricted to the upper layers of waters through which light can penetrate. The depth of photic zone varies depending on such local conditions as latitude, season, and particularly the turbidityof the water. Generally, the photosynthetic activity is confined to the upper 50to 125 m.Carbon di oxide is available largely from HCO3, although some gaseous carbondioxide is available.

Salinity:

The degree of salinity in natural waters ranges from zero in freshwater to saturation in salt lakes. A distintive charecteristic of sea water is its high salt content, which is remarkably constent. The

concentration of dissolved salt varies between 33 and 37 g/kg water. The principal salt are the chlorides, sulfates, and carbonates of sodium, potassium, calcium and magnesium. The concentration of salts is usually less in shallow offshore regions and near river mouths. Most marine microorganisms are halophilic; they grow best at salt concentrations of 2.5 to 4.0 percent, whereas those from lakes and rivers are salt sensitiveand do not grow at a salt concentration of more than 1 percent.

Turbidity:

There is marked variation in the clarity of surface waters. The Adriatic Sea is sparkling clear at great depths, whereas some near-shore rivers are often turbid. The suspended material responsible for the turbidity includes (1) particles of mineral material which originate from land; (2) detritus, predominantly particulate organic material, such as cellulose, hemicellulose, and chitin fragments; and (3) suspended microorganism. Many species of marine bacteria charecteristically grow while attached to a solid surface and are called epibacteria or periphytes.

Hydrogen ion concentration:

Aquatic microorganisms, in general, can be grown at pH 6.5 to 8.5. the pH of the sea is 7.5 to 8.5. Optimum growth of most marine species is obtained on media adjusted to pH 7.2 to 7.6. Lakes and rivers may show a wider range in pH depening upon local conditions.

Organic and Inorganic Constituents:

The quantity and type of inorganic and organic materials present in the aquatic environment are important in determining the microbial flora. Nitrate and phosphates are important inorganic constituents, particularly for the growth of algae. Organic compounds are required for the growth of saprophytic bacteria and fungi.

Techniques for the study of aquatic microorganisms:

Numerous problems are associated with attempts to charecterize the microbial flora of aquatic environments.
This is particularly true of samples from the open sea. Among these problems are the following:

Many aquatic microorganisms will not grow on the usual laboratory media such as nutrient agar or nutrient broth and consequently cannot be isolated. It is generally acknowledged that the estuaries and oceans contain a large number of microbial species that await discovery.

A high percentage of aquatic bacteria have a natural affinity to grow attached to solid surfaces, either on particulate material or on large organisms.

During the time which elapse between sample collection and transport back to the home-based laboratory, there is a lose of viability of many organisms. Accordingly, a laboratory-equipped ship is desirable for on-location culturing of specimens. This is a very costly facility.

The collection of samples from the depth of the estuary or ocean requires specialized sampling equipment.

Routine dependable techniques are not available for isolation of aquatic viruses.

A variety of procedures is used for the microbiological examination of aquatic specimens. The choice of method is determined by the purpose of the examination, e.g.:

1) Microscopic examination for identification and enumeration of algae, bacteria, protozoa, and many fungi.

(a) Direct viable counting of physiologically responsive bacteria

(b) Detection of specific bacteria by means of epifluorescent microscopy and fluorescent antibody techniques

(c) Detection of epibacteria by the submerged-slide techniques

2) Isolation and/or enukeration of certain groups of bacteria, eg., *Escherichia, Pseudomonas, Flavobacterium, Proteus, and Vibrio.* Many species of bacteria can be cultured by the plate technique on the usual bacteiological media. The membrane-filter technique is applicable for the examination and cultivation of many bacteria from the aquatic

environments. It can also be used to separate different-size fractions of the aquatic microbial community.

3) Enrichment culture technique for isolation of specific physiological or metabolic types of microorgnisms

4) Measurement of total mass or biochemical activity.

zz (a) Biomass determination. Dry weight determination of cell mass.

(b) Carbon 14 uptake: supplementation of the carbondioxide, in water with radioactive carbondioxide supplied as radioactive sodium bicarbonate and measurement of 14C assimilated by cells.

(c) ATP synthesis: Measurement of amount of ATP as a function of the rate of microbial activity or total biomass

(d) Chlorophyll determination: in the case of algae one can make a measure of chlorophyll.

PRESERVATION OF MARINE CULTURES:

There are five major types of culture preservation, they are found to be Agar slant culture preservation method, Agar slant culture covered with oil (Parafin method), Saline suspension method,

Drying in vacuum and Freeze drying.

Agar slant culture preservation:

Prepare a suitable agar slant for the organism to be preserved. Aseptically inoculate the culture to be preserved in the slant in the appropriate environment. Incubate the culture in suitable temperature for suitable amount of time. Now the culture can be preserved for future. This culture has to be continuously sub cultured to avoid contamination.

Agar slant culture covered with oil (paraffin):

Prepare a suitable agar slant for the organism to be preserved. Aseptically inoculate the culture to be preserved in the slant in the appropriate environment. Incubate the culture in suitable temperature for suitable amount of time. After the organisms are grown in the agar, slants are covered with oil or paraffin for 1cm above the tip of the agar slant. Now the culture can be preserved for future. Before the usage of the culture again the oil frozen in the slant has to be melted.

Saline suspension method:

The culture samples are mixed with sodium chloride (1% salt solution) so that the culture can be preserved from various other Organisms.

#Very low temperature broth with 15% glycerol suspension is frozen and stored at 15-30 degree Celsius.

#Availability of liquid nitrogen(-196 degree Celsius) provides another main preserving stock culture.Cultures are frozen with protective agent in sealed ampule (glycerol/dimethane sulphoxide). Sealed culture is kept in liquid nitrogen refrigerator.

Drying in vacuum:

The cultures are dried over Calcium chloride in vacuum and stored in refrigerator.

Freeze Drying:

Microbial suspension is placed in small vials. Thin Film is frozen over the inside surface of the vial by rotating it in mixture of dry ice and alcohol/acetone at -78 degree celcius. Then the vials are immediately connected to high vacuum line.this dries the organism while still frozen.now the ampoules are sealed with small flame and stored for several years at 40 degree celcius. Preservation of toxins, sera, enzymes, other biomaterials can also be done by this method.

Reviving cultures after preservation by this method:

Reviving the culture can be done by breaking the vial aseptically and add suitable state medium. Now you can incubate and use it for further transfers.

USES:

Permits maintainance of longer number of cultures without any variation in its characteristics.

It has reduced danger of contamination.

ENUMERATION (Total and Viable count):

The difference between total and viable count method is to determine the count of all cells ie., dead and alive cells is total cell count whereas the viable count estimate the number of viable or live cells only capable of growing into distinct colonies.

Direct Counting Method:

Direct counting method involves the use of immunofluorescence and epifluorescence adaptation of cell labeling used in conjunction with cytometry. Microscopy is a very old technique.

Reporter Assays:

Reporter assay is depended on population of microbes through metabolic activity. The results of this assay is examined by conductance, colorimetry, adenosine triphosphate(ATP) and it turbidity.

Slide and Cover slip Method:

The slide and cover slip method mainly involves slide and the cover slip for the assay. Here the number of microbes in edge is multiplied by dilution factor of the sample to get the number of original cell present in the sample.

Petridish Count:

In this method the sample is diluted to a point and inoculated in the media. These inoculated petridishes are kept in for incubation for suitable time period for the colonial growth. Now the number of colonies are counted and multiplied by its dilution factor to give number of cells in original sample. Results are given in colony forming unit (CFU) per ml (CFU/ML).

Total count: (Standard plate method)

Total count method involves all cells which includes bacteria, yeast and mold. Pour plate method is done for counting the total number of the cells in the given sample. The known volume of water is mixed with molten yeast – malt extract agar. The mixture is incubated in two set, one set of sample is incubated at 37 degree Celsius for 24 hours and the other set is incubated at 22 degree Celsius for 3days. The first set of samples

has less growth and the second set has more growth. This is because of the difference in the organisms present.

Direct and indirect count:

The direct count method is done with the help of Haemocytometer. The center – etched grids are examined. Accurate count should be from 40-70 cells in 1mm square. This requirement is not met, necessary adjustment by dilution or concentration is necessary.

Indirect count method mainly involves colorimeter. The Microorganisms grow well after incubation and become turbid. So this can be determined using colorimeter. Increase in optical density also increase in the number of microbes.

Coulter counter method measures the variation of conductivity of solution as bacteria passes through narrow group

The advantage of this method is it is completely automated and the disadvantage is that it cannot differentiate dead and live organisms.

Viable count:

The viable count method is the method where the viable colonies/cells are counted under favourable growth condition. Here the techniques like pour plate and spread plate methods can be used. Serial dilution method can also be followed, where it is little lenghthy process.

Disadvantages:

Time consuming

Takes long time of incubation period

Clumping of cells which leads to incorrect result

Some cases like too many/few colonies result will be correct

One of the advantage is it is very sensitive.

Isolation of cultures can be done using spread plate and streak plate method.

Identification of microorganism:

The morphological, physiological and biochemical characteristics can be identified,

Morphological identification:

The visible charecteristics of a colony is the morphological identification. This is not the reliable way to identify bacteria because different types of bacteria have similar colony morphology.

Form: circular, irregular, filamentous, rhizoid etc…

Elevation: Raised, convex, flat, umbonate, crateriform etc…

Margin: Entire, Undulate, Filiform, curled, lobate etc…

Physiological identification:

Gram's reaction, endospores, motility, metachromatic granules, aerobic/anaerobic, pH, temperature etc…

Biochemical test:

Gelatin hydrolysis, Caesin hydrolysis, Urea hydrolysis, Tryptophan hydrolysis, Lipid hydrolysis, Lecithin hydrolysis, Amylose hydrolysis, Pectin hydrolysis, DNA hydrolysis, catalase/cytochrome oxidase, Nitrate reduction test, Methyl red test, Acetone production etc…

Membrane filtation technique:

International and national collection centers:

The cultures are collected in 68 countries in about 587 culture collection centers. In India there are 21 culture collection centers. Only very few culture collection centers for marine microbiolology are available. Some of them are as below.

Culture Collection Centers in India

No.	Culture Collection Center	Remarks	Cultures Preserved
1	Anaerobic Bacterial Resource CenterDepartment of Plant Sciences, University of Hyderabad, Andhra PradeshTel: (91) 40-23134502E-mail: chvrsl@uohyd.ernet.in r449@sify.com		More than 210 bacterial strains are available.
2	Biological Nitrogen Fixation Project Colege of AgricultureCorrespondent: Prof. P. L. PatilPune 411005, Maharashtra, India		
	Culture Collection, Department of Microbiology, **Bose Institute**Prof. A. K.		

3	Mishra93/1 Acharya Prafulla Chandra,Calcutta,West Bengal,700009, India		
4	Culture Collection, Microbiology and Cell Biology Laboratory InstitutionIndian Institute of ScienceCorrespondent: Prof. G. Ramananda RaoAddress: Bangalore,Karnatka,560012 India		Bacteria 214Fungi 78Yeasts 43Cell lines: animal 10 Viruses: animal 3 Viruses: bacteria 11
5	Food and Fermentation Technology Division, University of MumbaiInstitution Dept. of Chemical TechnologyCorrespondent Assoc.Prof. P. R. KulkarniAddress: Nathalal Parekh Marg,Mumbai, Maharashtra,400019, India Tel: (91) 22-4145616 Fax: (91) 22-4145614 E-mail: prk@fft.udct.ernet.in		Bacteria 35Fungi 20Yeasts 05Algae 03
6	Goa University Fungus Culture Collection and Research Unit Correspondent: Dr. Nandkumar Kamat, Department of Botany, Goa University, Taleigao, Goa,Goa,403206, IndiaTelephone: (91) 0832-6519349Telephone: (91) 0832-423889629Fax: (91) 0832-2451184		Algae: 01Bacteria: 200Fungi: 1000Yeasts: 300

	E-mail: nandkamat@gmail.com E-mail: nkamat@unigoa.ac.in Web: www.unigoa.ac.in		Archaea: 30
7	Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, IndiaCorrespondent: Dr. Prameeladevi T. Telephone: (91)-9871980304E-mail: prameelancha@yahoo.co.inWeb: http://www.iari.res.in/		Total strains – 3800
8	Gujarat Biodiversity Genebank of GSBTM , GOG**Dr. Snehal Bagatharia** Sector Specialist – Agriculture Biotechnology ssabtm@gujarat.gov.in Direct line: +91 79 232 52165 **Dr. Madhavi Joshi** Sector Specialist – Pharma & Healthcare sspbtm@gujarat.gov.in Direct Line: +91 79 232 52168	Main focus of GSBTM is to preserve the various microorganisms in pure form isolated from diverse environment and provide support to academic institutes and companies in the direction of microbial research by preserving their microorganism, by providing them microbial culture in pure form for research purpose and by providing the services for molecular identification of	

		microbes.	
9	MACS Collection of Microorganisms MACS-Aghargar Research InstituteCorrespondent: Prof. K. M. Paknikar G.G.Agarkar Road,Pune,,411 004Country IndiaTelephone: (91) 20-5654357 Telephone: (91) 20-5653680 Fax: (91) 20-5651542 E-mail: paknikar@vsnl.com		Bacteria – 256Fungi – 08
10	Marathwada Agricultural universityCollection of Insect Pathogens, Dept. of EntomologyCorrespondent: Prof.		
	V. M. Pawar		
11	Microbial Culture CollectionNational Centre for Cell ScienceCorrespondent: Pro. Yogesh ShoucheUniversity of Pune Campus, Ganeshkhind, Pune 411007, Maharashtra, India Phone: +91-20-25708237, Fax:+91-20-25692259 E-mail: yogesh@nccs.res.in E-mail: yogesh.shouche@gmail.com Web: http://www.nccs.res.in/mcc/index.html	Free to Academic Culture Collections within networkExchange with Recognized culture collectionsFee of INR 700 for Academic Institutions (Freeze dried ampoules) and INR 2000 for industries	Bacteria 135488Fungi 41
	Microbial Type Culture Collection and Gene Bank (MTCC) Institute of Microbial	The MTCC is a modern facility housed at	

12	Technology (IMTECH) Correspondent: Prof. Chakrabarti TapanSector 39-A, Chandigarh-160036 (India) Phone (91-172) 263 66 80 to 94 Fax: (91-172) 269 05 85, 269 06 3 E.mail: curator@imtech.res.in Web: ***www.imtech.res.inhttp://mtcc.imtech.res.in***	Microbial Technology (IMTECH), Chandigarh. The MTCC was recognized by the World Intellectual Property Organization (WIPO), Geneva, Switzerland as an International Depository Authority (IDA) on 4 October,	Bacteria 1124Fungi 1245 Yeasts 575Plasmids 85
		2002, thus becoming the first IDA in India, seventh in Asia and thirty-fourth in the world.	
13	National Bureau of Agriculturally Important Microorganisms (NBAIM), Indian Council of Agricultural Research (ICAR), Kushmaur, P.B. No. 6, Kaithauli, Mau Nath Bhanjan 275 101 Uttar Pradesh, IndiaWeb: www.nbaim.org.in	The NBAIM culture collection is recognized as National Repository by Biodiversity Authority of India.Cultures are available:for Research Organization and Academia : 400.00 for Industry : 5000.00	Bacteria, filamentous fungi, yeasts, Actinomycetes
	National Centre for Cell Sciences, Pune	The National Centre for Cell Sciences, Pune (NCCS) was established	

14	(NCCS) University of Pune Campus, Ganeshkhind, Pune 411007, Maharashtra, India Phone: +91-20-25708000 Fax:+91-20-25692259 Gram: ATCELLWeb: www.nccs.res.in	as a National Repository of Animal Cell Culture with a mandate of basic research, teaching & training, and as a national repository for cell lines/ hybridomas	Animal Cell Cultures
		etc. It also conducts manpower development in animal tissue culture through training programmes / workshops and extends infrastructural facilities to researchers and institutions in biochemical sciences.	
15	National Collection of Dairy Cultures National Dairy Research Institute (Karnal)D. M. Division, National Dairy Research Institute, ,KARNAL, Haryana, 132001, IndiaTel: (91) 184-2259008Tel: (91) 184-2259198 Fax: (91) 184-2250042 E-mail: rsndri@gmail.com E-mail: registrar.ndri@gmail.com Web: http://www.ndri.res.in/		Bacteria 400Fungi 15Yeasts 20Dairy Starter Cultures 25

16	National Collection of Industrial MicroorganismsNational Chemical Laboratory (NCL) (CSIR)Correspondent Assoc. Prof. D.V. GokhaleAddress: Dr. Homi Bhabha Road, Pune, Maharashtra, 411 008, India Tel: (91) 20-25902670 Tel: (91) 20-25902454 Fax: (91) 20-25902671 E-mail: ncim@ncl.res.in Web: http://www.ncl-india.org/ncim/		Algae 15Bacteria 1400Fungi 950Yeasts 600
17	National Facility for Marine CyanobacteriaBharatidasan UniversityCorrespondent Prof. Lakshmanan UMAPostal Address Palkalai Perur,Tiruchirappalli,Tamil Nadu,620 024, India Telephone: (91) 431-2407084 Fax: (91) 431-2407084 E-mail:lumaprabakar@yahoo.com		

	E-mail: harmarpraba@yahoo.com Web: http://www.nfmc.res.in		
18	National Fungal Culture Collection of IndiaMACS' Agharkar Research InstituteCorrespondent: Dr. Sanjay K. SinghAddress: G.G. Agarakar Road, Pune, Maharashtra, 411004, India Tel: (91) 20-25653680 Tel: (91) 20-25654357 Fax: (91) 20-25651542 E-mail: sksingh@aripune.org E-mail: singhsksingh@gmail.com Web:http://www.aripune.org/indexnfcci. html	Free to exchange with most recognized collection; MTCC, DSMZ, CBS, NBRC, USDA etc.Fee for Active culture: INR 300 to Non-Profit Organization (in India)Fee for Active culture: INR 1500 to Profit Organization (in India)	Fungi 2800

	NII Microbial Culture CollectionNIICC		

19	National Institute for Interdisciplinary Science and Technology (CSIR) Correspondent: Prof. Ashok PandeyAddress: Industrial Estate, Pappanamcode, Trivandrum, Kerala, 695019, IndiaTel: (91) 471-2515276 Tel: (91) 471-2515279 Fax: (91) 471-2491712 Fax: (91) 471-2495949 E-mail: ashokpandey56@yahoo.co.in E-mail: binodkannur@yahoo.com Web: http://www.niist.res.in	Free to Free to Selected academic and teaching activitiesExchange with Exchange with Other WFCC collectionsFee for Fee for From US $ 75.00	Bacteria 294Fungi 78Yeast 07
20	North Maharashtra Microbial Culture Collection CentreInstitution North Maharashtra UniversityCorrespondent Assoc.Prof. Satish PatilAddress PB. 80, Umavi nagar, Jalgaon, Maharashtra,425001, India Tel: (91) 257-2257421 Fax: (91) 257-2258403		

E-mail: drsatishnmcc@gmail.com

E-mail: satish.patil7@gmail.com

2 1	VISVA-BHARATI CULTURE COLLECTION OF ALGAEVISVA-BHARATI CENTRAL UNIVERSITYCorrespondent Prof. Jnanendra RathAddress: Assistant Professor, Department of Botany, Visva-Bharati University, Santiniketan, West Bengal, 731235, India Tel: (91) 947-4766362 E-mail: vbcca@visva-bharati.ac.in E-mail: jrath@visva-bharati.ac.in Web: http://www.visva-bharati.ac.in/	Algae 50

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc MB COURSE NAME: MARINE MICROBIOLOGY

COURSE CODE: 18MBP105A UNIT: I BATCH-2018-2020

Sl. No	QUESTIONS	OPTION A	OPTION B	OPTION C	OPTION D	ANSWER
1	Attachment of small particles or molecules to a larger particle by electric charge is called as	Adsorption	absorption	fixation	attachment	absorption
2	_____are organism which grows at high pressure rather than at atmospheric pressure.	Barophile	halophile	thermophile	neutrophil	Barophile
3	_____ is Mass of living matter present	biogroup	Biomass	biodiverse	bioaccumulation	Biomass
4	Particulate (organic) material which is only partly disintegrated is called as_____	demeris	divergent	detritus	debris	detritus
5	An organism which grows preferentially in high salinities.	Halophile	Barophile	Chemophile	Divergephile	Halophile
6	Living together of two organisms with mutual advantage and without losing their identity is called as	Antagonism	Commensalism	Symbiosis	Mutualism	Commensalism
7	Which were the investigators lived at the same time?	Koch and Pasteur	Darwin and Woese	Van Leeuenhoek and Ricketts	Berg and Hooke	Koch and Pasteur
8	The unifying feature of the archaea that distinguishes them from the bacteria is	habitats which are extreme environments with regard to acidity	absence of a nuclear membrane temperature	presence of a cell wall containing a characteristic outer membrane	cytoplasmic ribosomes that are 70S	habitats which are extreme environments with regard to acidity

9	An organism which grows preferentially in high salinities.	Halophile	Barophile	Chemophile	Divergephile	Halophile
10	Organisms can synthesize ATP by oxidative phosphorylation when they	ferment	oxidize glucose to pyruvate	pass electrons from the oxidation of chlorophyll through an electron transport system	pass electrons to oxygen through an electron transport system containing cytochromes	oxidize glucose to pyruvate
11	Organism grows with fuzzy-cottony appearance	Yeast	Mold	Fungi	Bacteria	Mold
12	Agar slants are covered with _____ in culture preservation Cultures are frozen with protective agents like	Oil glycerol	Parafin dimethane sulphoxide	Both a and b glyceraldehyde	Grease A and B	Both a and b A and B
13	The staphylococcal intoxication refers to presence of	an enterotoxin	neurotoxin	mycotoxin	All of these	an enterotoxin
14	The concept of putting microbes to help clean up the environment is called	pasteurization	bioremediation	fermentation	biolistics	bioremediation
15	If a canning procedure is not properly followed, which type of microbe is most likely to grow in the canned	Obligate Aerobe	Acidophile	Mesophile	Obligate Anaerobe	Obligate Aerobe

	food?					
16	Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate	water rich in organic carbon wastes but poor in phosphate	water that are anoxic	water rich in phosphate wastes but poor in organic carbon	none of the above	water rich in organic carbon wastes but poor in phosphate
17	A musty or muddy odor of the fish is attributed to	the growth of *Streptomyces species* in the mud at the bottom of the body of water	the mud at the bottom of the body of water	the growth of *Pseudomonas species* in the mud at the bottom of the body of water	none of the above	the growth of *Pseudomonas species* in the mud at the bottom of the body of water
18	The concept of putting microbes to help clean up the environment is called	pasteurization	bioremediation	fermentation	biolistics	bioremediation
19	The predominant kind of bacteria causing spoilage in fish at chilling temperature is	species of *Pseudomonas*	*Micrococcus*	*Bacillus*	E.coli	species of *Pseudomonas*
20	Preservation of foods by using salts and sugars works by	raising pH	lowering osmotic pressure	creating a hypertonic environment	creating a hypotonic environment	raising Ph
21	Which of the following bacteria lack a cell wall and are therefore resistant to penicillin? Chemotaxis is a phenomenon of	Cyanobacteria swimming away of bacteria	Mycoplasmas swimming towards bacteria	Bdellovibrios swimming away or towards of bacteria in	Spirochetes Swimming in media	Mycoplasmas swimming away or towards of bacteria in presence of

				presence of chemical compound		chemical compound
22	The symptoms such as nausea and dehydration is caused by _____	*Shigella sonnei*	*Yersinia*	*Arizona*	*E.coli*	*Shigella sonnei*
23	The structure responsible for motility of bacteria is	pilli	flagella	sheath	capsules	flagella
24	_____ group of bacteria grows in high pressure.	Halophiles	thermophiles	Basophiles	psychrophiles	Basophiles
25	The group of gram positive bacteria having high G+C contents are called as	cyanobacteria	Nanobacteria	Firmicutes	Actinobacteria	Actinobacteria
26	_____ group of bacteria grows in high temperature	Halophiles	Basophiles	thermophiles	psychrophiles	thermophiles
27	The group of gram positive bacteria having low G+C contents are called as	cyanobacteria	Nanobacteria	Firmicutes	Actinobacteria	Firmicutes
28	BGA expanded as	Blue Green Algae	Blue Grown Algae	Blue non Grown Algae	Brown Green Algae	Blue Green Algae
29	Bacteria are _____organisms	Obligate	single celled	multicellular	seen by naked eyes.	single celled
30	_____ group of bacteria grows in high temperature	Halophiles	Basophiles	thermophiles	psychrophiles	thermophiles
31	Who is father of Marine Microbiology?	Leewenhoek	Zobell	Edward Jenner	Louis Pasteur	Zobell
32	Strain means_____	dye	Agent	Bacteria	organisms	organisms

33	Prokayotic ribosomes are made up of _____ subunits The time required to kill 90% of the microorganisms in a sample at a specific temperature is the	Two decimal reduction time	Three thermal death point	Five F value	ten D value	Two decimal reduction time
34	The etiological agent of Arizona infection is ____	*Vibrio*	*E. coli*	*Arizona*	*Streptococcus*	*Arizona*
35	Cyanobacteria have	a gram-positive cell wall	a gram-negative cell wall	no cytoplasm	No cell wall	a gram-positive cell wall
36	The unifying feature of the archaea that distinguishes them from the bacteria is	habitats which are extreme environments with regard to acidity	absence of a nuclear membrane temperature	presence of a cell wall containing a characteristic outer membrane	cytoplasmic ribosomes that are 70S	habitats which are extreme environments with regard to acidity
37	Suppose a eukaryotic cell had a mutation that prevented the production of cytochrome c. As a result of this mutation, which of the following processes would not occur?	Cellular respiration	Photosynthesis	Mitosis	Cell wall synthesis	Photosynthesis
38	In cellular metabolism, O2 is used	to provide electrons for photophosphorylation	in glycolysis	as a terminal electron acceptor	in the Krebs cycle	to provide electrons for photophosphorylation
39	The bacteria most often involved in the spoilage of fish are	Sarcina	Micrococcus or Bacillus species	Molds or yeasts	virus	Molds or yeasts

40	The red or pink color of the fish is generally caused from the growth of	part of the natural flora of the external slime of fishes and their intestinal contents	part of the natural flora of the internal slime of fishes only	no chage in structure	coliorms	part of the natural flora of the external slime of fishes and their intestinal contents
41	The Archaea include all of the following except	methanogens	halophiles	thermoacidophiles	cyanobacteria	cyanobacteria
42	The ocean contains _____ bacteria per milliliter (mL) of water.	105 - 107	107 – 109	106 - 107	105 – 108	107 – 109
43	A major cause of waterborne disease is the bacterium *Vibrio cholerae*, which causes_____	Cholera	dysentry	diarhoea	vomiting	Cholera
44	_____ causes diarrhea, urinary tract infections, bacteremia, and meningitis.	*Vibrio cholerae*	*E. coli*	*Salmonella typhi*	*Serratia*	*Salmonella typhi*
45	When the _____ is ingested by drinking, the mature adult spreads in the human host where it reproduces just below the skin.	copepod	coliforms	plankton	gastropod	copepod
46	Which of the following is a characteristic unique to the ciliates?	use flagella	Presence of both a macronucleus and several micronuclei	no cilia no flagella	Possess a light-detecting eye spot	Presence of both a macronucleus and several micronuclei
47	Suppose a eukaryotic cell had a mutation that prevented the	Cellular respiration	Photosynthesis	Mitosis	Cell wall synthesis	Photosynthesis

	production of cytochrome c. As a result of this mutation, which of the following processes would not occur?					
48	Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate	water rich in organic carbon wastes but poor in phosphate	water that is anoxic	water rich in phosphate wastes but poor in organic carbon	water rich in organic matters	water rich in organic carbon wastes but poor in phosphate
49	The cell walls of many gram positive bacteria can be easily destroyed by the enzyme known as	lipase	lysozyme	pectinase	peroxidase	lysozyme
50	Bacteria reproduce by _____ mechanism	fission	own	fusion	Direct	fission
51	Bacteria are sensitive to _____	Interleukins	Interferons	Antibiotics	Antitumours	Antibiotics
52	_____media is used for cultivation of bacteria	Nutrient agar	MacConkey agar	EMB agar	MHA	Nutrient agar
53	Single bacteria will form a _____ colony	Multiple	Single	No	infinite	Single
54	Which instrument is used for sterilization above 100° C	Flame	Autoclave	Filters	Desiccators	Autoclave
55	_____ is the first phase in growth curve	Log	Lag	stationary	death	Lag
56	The last step in synthesis of peptidoglycan is	attachment of a peptide to muramic acid	attaching two amino acids to form a cross-link	attachment of a portion of peptidoglycan to a membrane lipid	binding of penicillin to a membrane protein	attaching two amino acids to form a cross-link

57	Cytoplasmic inclusions exclude	ribosomes	mesosomes	fat globules	flagella	flagella
58	The cocci which forms a bunch and irregular pattern are	Staphylococci	diplococcic	Tetracocci	Streptococci	Staphylococci
59	Chemotaxis is a phenomenon of	swimming away of bacteria	swimming towards bacteria	swimming away or towards of bacteria in presence of chemical compound	Swimming in broth.	swimming away or towards of bacteria in presence of chemical compound
60	In vacuum drying, Organisms are dried over	Calcium chloride	Calcium carbonate	Sodium chloride	All the above	Calcium chloride

Unit II

Syllabus

Extremophiles: Thermopiles, basophiles, halophiles, psychrophiles, acid – alkalinophiles, oligotroph, toxitolerant, xerotolerant, endolith – Extremophiles and their environment, biodiversity. Genomics of extremophiles, phylogeny of extremophiles, 16S RNA classification in mitochondrial DNA genome, RAPD, RFLP studies.

Temperature ranges of microorganisms

Microorganisms can be grouped into broad (but not very precise) categories, according to their temperature ranges for growth.

Psychrophiles (cold-loving) can grow at 0°C, and some even as low as -10°C; their upper limit is often about 25°C.

Mesophiles grow in the moderate temperature range, from about 20°C (or lower) to 45°C.

Thermophiles are heat-loving, with an optimum growth temperature of 50° or more, a maximum of up to 70°C or more, and a minimum of about 20°C.

Hyperthermophiles have an optimum above 75°C and thus can grow at the highest temperatures tolerated by any organism. An extreme example is the genus *Pyrodictium*, found on geothermally heated areas of the seabed. It has a temperature minimum of 82°, optimum of 105° and growth maximum of 110°C.

It must be stressed that the temperature ranges for the groupings above are only approximate. For example, we would use different criteria to classify prokaryotes and eukaryotes. The upper temperature limit for growth of any thermophilic eukaryotic organism is about 62-65oC. And the upper limit for any photosynthetic eukaryote is about 57o - for the red alga Cyanidium caldarium, which grows around hot springs and has a temperature optimum of 45oC. In contrast to this, some unicellular cyanobacteria can grow at up to 75oC, and some non-photosynthetic prokaryotes can grow at 100oC or more.

Below, we consider two major types of thermophile - the microbes that grow in geothermal sites, and those that grow in "self-heating" materials such as composts. However, some very recent reports suggest that these different types of environment can share some common organisms.

Many of the prokaryotes that grow in the most extreme environments are archaea - a group that is clearly distinguishable from both the present-day bacteria and the eukaryotes. There is little doubt that many of them still remain to be discovered and described, but this is a difficult field of research because of the problem of reproducing their natural growth conditions in a laboratory environment. Members of the genus Sulfolobus (archaea) are among the best-studied hyperthermophiles. They are commonly found in geothermal environments, with a maximum growth temperature of about 85-90o, optimum of about 80o and minimum of about 60oC. They also have a low pH optimum (pH 2-3) so they are termed thermoacidophiles. Sulfolobus species gain their energy by oxidising the sulphur granules around hot springs, generating sulphuric acid and thereby lowering the pH.

The study of extreme environments has considerable biotechnological potential. For example, the two thermophilic species Thermus aquaticus and Thermococcus litoralis are used as sources of the enzyme DNA polymerase, for the polymerase chain reaction (PCR) in DNA fingerprinting, etc. The enzymes from these organisms are stable at relatively high temperatures, which is necessary for the PCR process which involves cycles of heating to break the hydrogen bonds in DNA and leave single strands that can be copied repeatedly. Another thermophile, Bacillus stearothermophilus (temperature maximum 75oC) has been grown commercially to obtain the enzymes used in 'biological' washing powders.

The microbiology of hot springs and geothermal vents

Hot springs and geothermal vents are found in several parts of the world, but the largest single concentration is in Yellowstone National Park, USA. The images below show how some thermophilic prokaryotes (bacteria and archaea) are specially adapted to grow in these environments. In each case we find a zonation of microorganisms according to their temperature optima. Often these organisms are coloured, due to the presence of pigments (blue-green of cyanobacteria, red of red algae or purple bacteria) or carotenoid pigments (yellows and browns of some archaea).

A **thermophile** is an organism—a type of extremophile—that thrives at relatively high temperatures, between **41 and 122 °C (106 and 252 °F)**. Many thermophiles are archaea. Thermophilic eubacteria are suggested to have been among the earliest bacteria.

Thermophiles are found in various geothermally heated regions of the Earth, such as hot springs like those in Yellowstone National Park (see image) and deep sea hydrothermal vents, as well as decaying plant matter, such as peat bogs and compost.

Thermophiles can survive at high temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures.

The enzymes in thermophiles necessarily function at high temperatures. Some of these enzymes are used in molecular biology, for example, heat-stable DNA polymerases for PCR), and in washing agents.

"Thermophile" is derived from the Greek: θερμότητα (*thermotita*), meaning heat, and Greek: φίλια (*philia*), love.

Classification

Thermophiles can be classified in various ways. One classification sorts these organisms according to their optimal growth temperatures:

Simply thermophiles: 50–64 °C

Extreme thermophiles 65–79 °C

Hyperthermophiles 80 °C and beyond, but not < 50 °C.

In a related classification, thermophiles are sorted as follows:

Obligate thermophiles (also called extreme thermophiles) require such high temperatures for growth, whereas

Facultative thermophiles (also called moderate thermophiles) can thrive at high temperatures, but also at lower temperatures (below 50 °C (122 °F)).

Hyperthermophiles are particularly extreme thermophiles for which the optimal temperatures are above 80 °C (176 °F).

A colony of thermophiles in the outflow of Mickey Hot Springs, Oregon, the water temperature is approximately 60 °C (140 °F).

Many of the hyperthermophiles Archea require elemental sulfur for growth. Some are anaerobes that use the sulfur instead of oxygen as an electron acceptor during cellular respiration. Some are lithotrophs that oxidize sulphur to sulfuric acid as an energy source, thus requiring the microorganism to be adapted to very low pH (i.e., it is an acidophile as well as thermophile). These organisms are inhabitants of hot, sulfur-rich environments usually associated with volcanism, such as hot springs, geysers, and fumaroles. In these places, especially in Yellowstone National Park, zonation of microorganisms according to their temperature optima occurs. Often, these organisms are colored, due to the presence of photosynthetic pigments.

Thermophile versus mesophile

Thermophiles can be discriminated from mesophiles from genomic features. For example, the GC-content levels in the coding regions of some signatures genes were consistently identified as correlated with the temperature range condition when the association analysis was applied to mesophilic and thermophilic organisms regardless of their phylogeny, oxygen requirement, salinity, or habitat conditions.

Gene transfer and genetic exchange

Sulfolobus solfataricus and *Sulfolobus acidocaldarius* are hyperthermophilic archaea. When these organisms are exposed to the DNA damaging agents UV irradiation, bleomycin or mitomycin C, species-specific cellular aggregation is induced. In *S. acidocaldarius*, UV-induced cellular aggregation mediates chromosomal marker exchange with high frequency. Recombination rates exceed those of uninduced cultures by up to three orders of magnitude. Frols et al. and Ajon et al.(2011) hypothesized that cellular aggregation enhances species-specific DNA transfer between *Sulfolobus* cells in order to provide increased repair of damaged DNA by means of homologous recombination. Van Wolferen et al., in discussing DNA exchange in the hyperthermophiles under extreme conditions, noted that DNA exchange likely plays a role in repair of DNA via homologous recombination. They suggested that this process is crucial under DNA damaging conditions such as high temperature. Also it has been suggested that DNA transfer

in *Sulfolobus*may be a primitive form of sexual interaction similar to the more well-studied bacterial transformation systems that are associated with species-specific DNA transfer between cells leading to homologous recombinational repair of DNA damage [see Transformation (genetics)].

BASOPHILLS

Basophils are a type of white blood cells. Basophils are the least common of the granulocytes, representing about 0.5 to 1% of circulating white blood cells.[3] However, they are the largest type of granulocyte. They are responsible for inflammatory reactions during immune response, as well as in the formation of acute and chronic allergic diseases, including anaphylaxis, asthma, atopic dermatitis and hay fever. They can perform phagocytosis (cell eating), produce histamine and serotonin that induce inflammation, and heparin that prevents blood clotting although there are less than that found in Mast cell granules. It used to be thought that basophils that have migrated from blood into their resident tissues (connective tissue) are known as mast cells, but this is no longer thought to be the case.

Basophils were discovered in 1879 by German physician Paul Ehrlich, who one year earlier had found a cell type present in tissues that he termed *mastzellen* (now mast cells) Ehrlich received the 1908 Nobel Prize in Physiology or Medicine for his discoveries.

The name comes from the fact that these leukocytes are basophilic, i.e., they are susceptible to staining by basic dyes, as shown in the picture

Structure

Basophils contain large cytoplasmic granules which obscure the cell nucleus under the microscope when stained. However, when unstained, the nucleus is visible and it usually has two lobes. The mast cell, another granulocyte, is similar in appearance and function. Both cell types store histamine, a chemical that is secreted by the cells when stimulated. However, they arise from different branches of hematopoiesis, and mast cells usually do not circulate in the blood stream, but instead are located in connective tissue. Like all circulating granulocytes, basophils can be recruited out of the blood into a tissue when needed.

Function

Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents

blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. They can be found in unusually high numbers at sites of ectoparasiteinfection, e.g., ticks. Like eosinophils, basophils play a role in both parasitic infections and allergies They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defense and allergy. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behavior of T cells and mediate the magnitude of the secondary immune response.

CD200

Basophil function is inhibited by CD200. Herpesvirus-6, herpesvirus-7, and herpesvirus-8 produce a CD200 homolog which also inhibits basophil function. This suggests that basophils may play a role in the immune response to these viruses.

Secretions

Basophils arise and mature in bone marrow. When activated, basophils degranulate to release histamine, proteoglycans (e.g. heparin and chondroitin), and proteolytic enzymes(e.g. elastase and lysophospholipase). They also secrete lipid mediators like leukotrienes (LTD-4), and several cytokines. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation. Recent evidence suggests that basophils are an important source of the cytokine, interleukin-4, perhaps more important than T cells. Interleukin-4 is considered one of the critical cytokines in the development of allergies and the production of IgE antibody by the immune system. There are other substances that can activate basophils to secrete which suggests that these cells have other roles in inflammation.

The degranulation of basophils can be investigated *in vitro* by using flow cytometry and the so-called basophil-activation-test (BAT). Especially, in the diagnosis of allergies including of drug reactions (e.g. induced by contrast medium), the BAT is of great impact.

Basopenia (a low basophil count) is difficult to demonstrate as the normal basophil count is so low; it has been reported in association with autoimmune urticaria (a chronic itching condition). Basophilia is also uncommon but may be seen in some forms of leukaemia or lymphoma.

Clinical significance

Immunophenotyping

Basophils of mice and humans have consistent immunophenotypes,
including FcεRI+, CD123, CD49b(DX-5)+, CD69+, Thy-1.2+, 2B4+, CD11bdull, CD117(c-kit)-, CD24-, CD19-, CD80-, CD14-, CD23-, Ly49c-, CD122-, CD11c-, Gr-1-, NK1.1-, B220-, CD3-, γδTCR-, αβTCR-, α_4 and β_4-integrin negative.

Recently, Heneberg proposed that basophils may be defined as the cellular population positive for CD13, CD44, CD54, CD63, CD69, CD107a, CD123, CD164, CD193/ CCR3, CD203c, TLR-4, and FcεRI. When activated, some additional surface markers are known to be upregulated (CD13, CD107a, CD164), or surface-exposed (CD63, and the ectoenzyme CD203c).

PSYCHROPHILLS

Psychrophiles or **cryophiles** (adj. psychrophilic or cryophilic) are extremophilic organisms that are capable of growth and reproduction in low temperatures, ranging from −20 ° to +10 °C. They are found in places that are permanently cold, such as the polar regions and the deep sea. They can be contrasted with thermophiles, which are organisms that thrive at unusually high temperatures. Psychrophile is Greek for 'cold-loving'.

Many such organisms are bacteria or archaea, but some eukaryotes such as lichens, snow algae, fungi, and wingless midges, are also classified as psychrophiles.

Habitat

The cold environments that psychrophiles inhabit are ubiquitous on Earth, as a large fraction of our planetary surface experiences temperatures lower than 15 °C. They are present in permafrost, polar ice, glaciers, snowfields and deep ocean waters. These organisms can also be found in pockets of sea ice with high salinity content. Microbial activity has been measured in soils frozen below −39 °CIn addition to their temperature limit, psychrophiles must also adapt to other

extreme environmental constraints that may arise as a result of their habitat. These constraints include high pressure in the deep sea, and high salt concentration on some sea ice.

Adaptations

Psychrophiles are protected from freezing and the expansion of ice by ice-induced desiccation and vitrification (glass transition), as long as they cool slowly. Free living cells desiccate and vitrify between −10 °C and −26 °C. Cells of multicellular organisms may vitrify at temperatures below −50 °C. The cells may continue to have some metabolic activity in the extracellular fluid down to these temperatures, and they remain viable once restored to normal temperatures.

They must also overcome the stiffening of their lipid cell membrane, as this is important for the survival and functionality of these organisms. To accomplish this, psychrophiles adapt lipid membrane structures that have a high content of short, unsaturated fatty acids. Compared to longer saturated fatty acids, incorporating this type of fatty acid allows for the lipid cell membrane to have a lower melting point, which increases the fluidity of the membranes. In addition, carotenoids are present in the membrane, which help modulate the fluidity of it.

Antifreeze proteins are also synthesized to keep psychrophiles' internal space liquid, and to protect their DNA when temperatures drop below water's freezing point. By doing so, the protein prevents any ice formation or recrystallization process from occurring.

The enzymes of these organisms have been hypothesized to engage in a activity-stability-flexibility relationship as a method for adapting to the cold; the flexibility of their enzyme structure will increase as a way to compensate for the freezing effect of their environment.

Certain cryophiles, such as Gram-negative bacteria *Vibrio* and *Aeromonas* spp., can transition into a viable but nonculturable (VBNC) state. During VBNC, a micro-organism can respirate and use substrates for metabolism – however, it cannot replicate. An advantage of this state is that it is highly reversible. It has been debated whether VBNC is an active survival strategy or if eventually the organism's cells will no longer be able to be revived. There is proof however it may be very effective – Gram positive bacteria Actinobacteria have been shown to have lived about 500,000 years in the permafrost conditions of Antarctica, Canada, and Serbia.

Psychrophiles include bacteria, lichens, fungi, and insects.

Among the bacteria that can tolerate extreme cold are *Arthrobacter* sp., *Psychrobacter* sp. and members of the genera *Halomonas*, *Pseudomonas*, *Hyphomonas*, and *Sphingomonas*.[11] Another example is *Chryseobacterium greenlandensis*, a psychrophile that was found in 120,000-year-old ice.

Umbilicaria antarctica and *Xanthoria elegans* are lichens that have been recorded photosynthesizing at temperatures ranging down to −24 °C, and they can grow down to around −10 °C. Some multicellular eukaryotes can also be metabolically active at sub-zero temperatures, such as some conifers; those in the *Chironomidae* family are still active at −16 °C.

Penicillium is a genus of fungi found in a wide range of environments including extreme cold.

Among the psychrophile insects, the Grylloblattidae or icebugs, found on mountaintops, have optimal temperatures between 1-4 °C. The wingless midge (Chironomidae) *Belgica antarctica* is the only insect, indeed the only terrestrial animal, endemic to Antarctica; it can tolerate salt, being frozen and strong ultraviolet, and has the smallest known genome of any insect. The small genome, of 99 million base pairs, is thought to be adaptive to extreme environments.

Psychrotrophic bacteria

Psychrotrophic bacteria are capable of surviving or even thriving in extremely cold environment. They provide an estimation of the product's shelf life, also they can be found in soils, in surface

and deep sea waters, in Antarctic ecosystems, and in foods. They are responsible for spoiling refrigerated foods.

Psychrotrophic bacteria are of particular concern to the dairy industry. Most are killed by pasteurization; however, they can be present in milk as post-pasteurization contaminants due to less than adequate sanitation practices. According to the Food Science Department at Cornell University, psychrotrophs are bacteria capable of growth at temperatures at or less than 7 °C (44.6 °F). At freezing temperatures, growth of psychrotrophic bacteria becomes negligible or virtually stops.

All three subunits of the RecBCD enzyme are essential for physiological activities of the enzyme in the Antarctic *Pseudomonas syringae*, namely, repairing of DNA damage and supporting the growth at low temperature. The RecBCD enzymes are exchangeable between the psychrophilic *P. syringae* and the mesophilic *E. coli* when provided with the entire protein complex from same species. However, the RecBC proteins (RecBCPs

and RecBCEc) of the two bacteria are not equivalent; the RecBCEc is proficient in DNA recombination and repair, and supports the growth of *P. syringae* at low temperature, while RecBCPs is insufficient for these functions. Finally, both helicase and nuclease activity of the RecBCDPs are although important for DNA repair and growth of *P. syringae* at low temperature, the RecB-nuclease activity is not essential in vivo.

Versus psychrotroph

In 1940, ZoBell and Conn stated that they had never encountered "true psychrophiles" or organisms that grow best at relatively low temperatures. In 1958, J. L. Ingraham supported this by concluding that there are very few or possibly no bacteria that fit the textbook definitions of psychrophiles. Richard Y. Morita emphasizes this by using the term *psychrotroph* to describe organisms that do not meet the definition of psychrophiles. The confusion between the terms *psychrotrophs* and *psychrophiles* was started because investigators were unaware of the thermolability of psychrophilic organisms at the laboratory temperatures. Due to this, early investigators did not determine the cardinal temperatures for their isolates. The similarity between these two is that they are both capable of growing at zero, but optimum and upper temperature limits for the growth are lower for psychrophiles compared to psychrotrophs. Psychrophiles are also more often isolated from permanently cold habitats compared to psychrotrophs. Although psychrophilic enzymes remain under-used because the cost

of production and processing at low temperatures is higher than for the commercial enzymes that are presently in use, the attention and resurgence of research interest in psychrophiles and psychrotrophs will be a contributor to the betterment of the environment and the desire to conserve energy.

Halophills

Halophiles are organisms that thrive in high salt concentrations. They are a type of extremophile organisms. The name comes from the Greek word for "salt-loving". While most halophiles are classified into the Archaea domain, there are also bacterial halophiles and some eukaryota, such as the alga *Dunaliella salina* or fungus *Wallemia ichthyophaga*. Some well-known species give off a red color from carotenoid compounds, notably bacteriorhodopsin. Halophiles can be found anywhere with a concentration of salt five times greater than the salt concentration of the ocean,

such as the Great Salt Lake in Utah, Owens Lake in California, the Dead Sea, and in evaporation ponds.

Classification

Halophiles are categorized as slight, moderate, or extreme, by the extent of their halotolerance. Slight halophiles prefer 0.3 to 0.8 M (1 .7 to 4.8% —seawater is 0.6 M or 3.5%), moderate halophiles 0.8 to 3.4 M (4.7 to 20%), and extreme halophiles 3.4 to 5.1 M (20 to 30%) salt content.[1] Halophiles require sodium chloride (salt) for growth, in contrast to halotolerant organisms, which do not require salt but can grow under saline conditions.

Lifestyle

High salinity represents an extreme environment to which relatively few organisms have been able to adapt and occupy. Most halophilic and all halotolerant organisms expend energy to exclude salt from their cytoplasm to avoid protein aggregation ('salting out'). To survive the high salinities, halophiles employ two differing strategies to prevent desiccation through osmotic movement of

water out of their cytoplasm. Both strategies work by increasing the internal osmolarity of the cell. In the first (which is employed by the majority of halophilic bacteria, some archaea, yeasts, algae and fungi), organic compounds are accumulated in the cytoplasm— osmoprotectants which are known as compatible solutes. These can be either synthesised or
accumulated from the environment.[2] The most common compatible solutes are neutral or zwitterionic, and include amino acids, sugars, polyols, betaines, and ectoines, as well as derivatives of some of these compounds.

The second, more radical, adaptation involves the selective influx of potassium (K^+) ions into the cytoplasm. This adaptation is restricted to the moderately halophilic bacterial order Halanaerobiales, the extremely halophilic archaeal family Halobacteriaceae, and the extremely halophilic bacterium *Salinibacter ruber*. The presence of this adaptation in three distinct evolutionary lineages suggests convergent evolution of this strategy, it being unlikely to be an ancient characteristic retained in only scattered groups or passed on through massive lateral gene transfer. The primary

reason for this is the entire intracellular machinery (enzymes, structural proteins, etc.) must be adapted to high salt levels, whereas in the compatible solute adaptation, little or no adjustment is required to intracellular macromolecules; in fact, the compatible solutes often act as more general stress protectants, as well as just osmoprotectants

Of particular note are the extreme halophiles or haloarchaea (often known as halobacteria), a group of archaea, which require at least a 2 M salt concentration and are usually found in saturated solutions (about 36% w/v salts). These are the primary inhabitants of salt lakes, inland seas, and evaporating ponds of seawater, such as the deep salterns, where they tint the water column and sediments bright colors. These species most likely perish if they are exposed to anything other than a very high-concentration, salt-conditioned environment. These prokaryotes require salt for growth. The high concentration of sodium chloride in their environment limits the availability of oxygen for respiration. Their cellular machinery is adapted to high salt concentrations by having charged amino acids on their surfaces, allowing the retention of water molecules around these components. They are heterotrophs that normally respire by aerobic means. Most halophiles are unable to survive outside their high-salt native environments. Indeed, many cells are so fragile that when placed in distilled water, they immediately lyse from the change in osmotic conditions.

Halophiles may use a variety of energy sources. They can be aerobic or anaerobic. Anaerobic halophiles include phototrophic, fermentative, sulfate-reducing, homoacetogenic, and methanogenic species.

The Haloarchaea, and particularly the family Halobacteriaceae, are members of the domain Archaea, and comprise the majority of the prokaryotic population in hypersaline environments. Currently, 15 recognised genera are in the family.[5] The domain Bacteria (mainly *Salinibacter ruber*) can comprise up to 25% of the prokaryotic community, but is more commonly a much lower percentage of the overall population At times, the alga *Dunaliella salina* can also proliferate in this environment.

A comparatively wide range of taxa has been isolated from saltern crystalliser ponds, including members of these genera: *Haloferax, Halogeometricum, Halococcus, Haloterrigena, Halorubrum, Haloarcula*, and *Halobacterium* However, the viable counts in these cultivation studies have been small when compared to total counts, and the numerical significance of these isolates has been unclear. Only recently has it become possible to determine the identities and relative abundances of organisms in natural populations, typically using PCR-based strategies that target 16S small subunit ribosomal ribonucleic acid (16S rRNA) genes. While comparatively few studies of this

type have been performed, results from these suggest that some of the most readily isolated and studied genera may not in fact be significant in the *in situ* community. This is seen in cases such as the genus *Haloarcula*, which is estimated to make up less than 0.1% of the *in situ* community, but commonly appears in isolation studies.

Genomic and proteomic signature

The comparative genomic and proteomic analysis showed distinct molecular signatures exist for environmental adaptation of halophiles. At the protein level, the halophilic species are characterized by low hydrophobicity, overrepresentation of acidic residues, underrepresentation of Cys, lower propensities for helix formation, and higher propensities for coil structure. The core of these proteins is less hydrophobic, such as DHFR, that was found to have narrower β - strandsAt the DNA level, the halophiles exhibit distinct dinucleotide and codon usage.

Examples

Halobacterium is a genus of the Archaea that has a high tolerance for elevated levels of salinity. Some species of halobacteria have acidic proteins that resist the denaturing effects of salts. *Halococcus* is a specific genus of the family Halobacteriaceae.

Some hypersaline lakes are a habitat to numerous families of halophiles. For example, the Makgadikgadi Pans in Botswana form a vast, seasonal, high-salinity water body that manifests halophilic species within the diatom genus *Nitzschia* in the family Bacillariaceae, as well as species within the genus *Lovenula* in the family Diaptomidae Owens Lake in California also contains a large population of the halophilic bacterium *Halobacterium halobium*.

Wallemia ichthyophaga is a basidiomycetous fungus, which requires at least 1.5 M sodium chloride for *in vitro* growth, and it thrives even in media saturated with salt. Obligate requirement for salt is an exception in fungi. Even species that can tolerate salt concentrations close to saturation (for example *Hortaea werneckii*) in almost all cases grow well in standard microbiological media without the addition of salt.

The fermentation of salty foods (such as soy sauce, Chinese fermented beans, salted cod, salted anchovies, sauerkraut, etc.) often involves halobacteria, as either essential ingredients or accidental contaminants. One example is *Chromohalobacter beijerinckii*, found in salted beans preserved in brine and in salted herring. *Tetragenococcus halophilus*is found in salted anchovies and soy sauce.

North Ronaldsay sheep are a breed of sheep originating from Orkney, Scotland. They have limited access to fresh water sources on the island and to their only food source is seaweed. They have adapted to handle salt concentrations that would kill other breeds of sheep.

LIGOTROPH

An **oligotroph** is an organism that can live in an environment that offers very low levels of nutrients. They may be contrasted with copiotrophs, which prefer nutritionally rich environments. Oligotrophs are characterized by slow growth, low rates of metabolism, and generally low population density.

The adjective *oligotrophic* may be used to refer to environments that offer little to sustain life, organisms that survive in such environments, or the adaptations that support

Plant adaptations

Plant adaptations to oligotrophic soils provide for greater and more efficient nutrient uptake, reduced nutrient consumption, and efficient nutrient storage. Improvements in nutrient uptake are facilitated by root adaptations such as nitrogen-fixing root nodules, mycorrhizae and cluster roots. Consumption is reduced by very slow growth rates, and by efficient use of low-availability nutrients; for example the use of highly available ions to maintain turgor pressure, with low-availability nutrients reserved for the building of tissues. Despite these adaptations, nutrient requirement typically exceed uptake during the growing season, so many oligotrophic plants have the ability to store nutrients, for example in trunk tissues, when demand is low, and remobilise them when demand increases.

Oligotrophic environments

Oligotrophs occupy environments where the available nutrients offer little to sustain life. The term "**oligotrophic**" is commonly used to describe terrestrial and aquatic environments with very low concentrations of nitrates, iron, phosphates, and carbon sources

Oligotrophs have acquired survival mechanisms that involve the expression of genes during periods of low nutrient conditions, which has allowed them to find success in various environments. Despite the capability to live in low nutrient concentrations, oligotrophs may find difficulty surviving in nutrient-rich environments.

A n t a r c t i c a

Antarctic life offers very little to sustain life as most organisms are not well adapted to live under nutrient-limiting conditions and cold temperatures (lower than 5 °C). As such, these environments display a large abundance of psychrophiles that are well adapted to living in an Antarctic biome. Most oligotrophs live in lakes where water helps support biochemical processes for growth and survival Below are some documented examples of oligotrophic environments in Antarctica:

Lake Vostok, a freshwater lake which has been isolated from the world beneath 4 km (2.5 mi) of Antarctic ice is frequently held to be a primary example of an oligotrophic environment. Analysis of ice samples showed ecologically separated microenvironments. Isolation of microorganisms from each microenvironment led to the discovery of a wide range of different microorganisms present within the ice sheet. Traces of fungi have also been observed which suggests potential for unique symbiotic interactions.The lake's extensive oligotrophy has led some to believe parts of lake are completely sterile. This lake is a helpful tool for simulating studies regarding extraterrestrial life on frozen planets and other celestial bodies.

Crooked Lake is an ultra-oligotrophic glacial lake with a thin distribution of heterotrophic and autotrophic microorganisms.[11] The microbial loop plays a big role in cycling nutrients and energy within this lake, despite particularly low bacterial abundance and productivity in these environments. The little ecological diversity can be attributed to the lake's low annual temperatures.Species discovered in this lake include *Ochromonas, Chlamydomonas, Scourfeldia, Cryptomonas, Akistrodesmus* competitive *falcatus,* and *Daphniopsis studeri* (a microcrustacean). It is proposed that low selection against *Daphniopsis studeri* has allowed the species to survive long enough to reproduce in nutrient limiting environments.

Australia

The sandplains and lateritic soils of southern Western Australia, where an extremely thick craton has precluded any geological activity since the Cambrian and there has been no glaciation to renew soils since the Carboniferous. Thus, soils are extremely nutrient-poor and most vegetation must use strategies such as cluster roots to gain even the smallest quantities of such nutrients as phosphorus and sulfur.

The vegetation in these regions, however, is remarkable for its biodiversity, which in places is as great as that of a tropical rainforest and produces some of the most spectacular wildflowers in the world. It is however, severely threatened by climate change which has moved the winter rain belt south, and also by clearing for agriculture and through use of fertilizers, which is primarily driven by low land costs which make farming economic even with yields a fraction of those in Europe or North America.

South America

An example of oligotrophic soils are those on white-sands, with soil pH lower than 5.0, on the Rio Negro basin on northern Amazonia that house very low-diversity, extremely fragile forests and savannahs drained by blackwater rivers; dark water colour due to high concentration
of tannins, humic acids and other organic compounds derived from the very slow decomposition of plant matter. Similar forests are found in the oligotrophic waters of the Patía River delta on the Pacific side of the Andes.

Ocean

In the ocean, the subtropical gyres north and south of the equator are regions in which the nutrients required for phytoplankton growth (for instance, nitrate, phosphate and silicic acid)
are strongly depleted all year round. These areas are described as oligotrophic and exhibit low surface chlorophyll. They are occasionally described as "ocean deserts".

Oligotrophic soil environments

The oligotrophic soil environments include agricultural soil, frozen soil etc. Various factors,
such as decomposition, soil structure, fertilization and temperature, can affect the nutrient-availability in the soil environments.
Generally, the nutrient becomes less available along the depth of the soil environment, because on the surface, the organic compounds decomposed from the plant and animal debris are consumed quickly by other microbes, resulting in the lack of nutrient in the deeper level of soil. In addition, the metabolic waste produced by the microorganisms on the surface also causes the accumulation of toxic chemicals in the deeper area. Furthermore, oxygen and water are important for some metabolic pathways, but it is difficult for water and oxygen to diffuse as the depth increases. Some factors, such as soil aggregates, pores and extracellular enzymes, may

help water, oxygen and other nutrients diffuse into the soil. Moreover, the presence of mineral under the soil provides the alternative sources for the species living in the

oligotrophic soil. In terms of the agricultural lands, the application of fertilizer has a complicated impact on the source of carbon, either increasing or decreasing the organic carbon in the soil.

Collimonas is one of the species that are capable of living in the oligotrophic soil. One common feature of the environments where *Collimonas* lives is the presence of fungi, because *Collimonas* have the ability of not only hydrolyzing the chitin produced by fungi for

nutrients, but also producing materials (e.g., *P. fluorescens* 2-79) to protect themselves from fungal infection. The mutual relationship is common in the oligotrophic environments.

Additionally, *Collimonas* can also obtain electron sources from rocks and minerals by weathering.

In terms of polar areas, such as Antarctic and Arctic region, the soil environment is considered as oligotrophic because the soil is frozen with low biological activities. The most abundant species in the frozen soil are Actinobacteria, Proteobacteria, Acidobacteria and Cyanobacteria, together with a small amount of archaea and fungi. Actinobacteriacan maintain the activity of their metabolic enzymes and continue their biochemical reactions under a wide range of low temperature. In addition, the DNA repairing machinery in Actinobacteria protects them from lethal DNA mutation at low temperature.

TOXITOLERANT AND XEROTOLERANT:

Geothermal Biodiversity

We are cataloguing New Zealand's unique terrestrial extremophile biodiversity and ecology. See the 1000 Springs Projec

One feature that makes New Zealand unlike any other place on Earth is the presence of a vast array of extreme environments in a small geographical area. These include volcanic crater lakes, steaming ground, deep subsurface coal seams, hot springs and boiling mud pools. The majority of these environments are found in the Taupō Volcanic Zone (TVZ) which extends from Mt Ruapehu to White Island.

Research has shown that these environments support microorganisms that thrive under extreme physical and chemical stresses that other life forms cannot withstand.

Researchers at GNS Science are interested in detailed taxonomic, genetic, metabolic, and ecological characterisation of these microbial biota. We seek to investigate the factors that influence biocomplexity within and across the systems, and to understand the biogeochemical processes catalysed by their microbial communities.

This research will provide information for:

Definition of these natural resources

Effective management of extreme ecosystems

Improved awareness of a unique ecosystem

New methods for characterising microbial biodiversity

Specialised capability in New Zealand

Springboard for scientific endeavour

Commercial opportunities

Examples of our research into New Zealand's microbial biodiversity include:

Extremophilic microorganisms at Champagne Pool, Waiotapu

The variations in microbial population structures in response to temperature variations at Inferno Crater, Waimangu.

1000 Spring Project.Research into the physical, chemical and microbiological diversity of 1000 hotsprings in the TVZ.

Acidophilic methanotrophs from the phylum *Verrucomicrobia*.

16sr RNA classification in mitochondria

16S ribosomal RNA (or 16S rRNA) is the component of the 30S small subunit of a prokaryotic ribosome that binds to the Shine-Dalgarno sequence. The genes coding for it are referred to as **16S rRNA gene** and are used in reconstructing phylogenies, due to the slow rates of evolution of this region of the gene. Carl Woeseand George E. Fox were two of the people who pioneered the use of 16S
rRNA in phylogenetics.

Multiple sequences of the 16S rRNA gene can exist within a single bacterium.

Functions

It has several functions:

Like the large (23S) ribosomal RNA, it has a structural role, acting as a scaffold defining the positions of the ribosomal proteins.

The 3' end contains the anti-Shine-Dalgarno sequence, which binds upstream to the AUG start codon on the mRNA. The 3'-end of 16S RNA binds to the proteins S1 and S21 known to be

involved in initiation of protein synthesis; RNA-protein cross-linking by A.P. Czernilofsky et al. (FEBS Lett. Vol 58, pp 281– 284, 1975).

Interacts with 23S, aiding in the binding of the two ribosomal subunits (50S+30S)

Stabilizes correct codon-anticodon pairing in the A site, via a hydrogen bond formation between the N1 atom of Adenine (*see image of Purine chemical structure*) residues 1492 and 1493 and the 2'OH group of the mRNA backbone

Universal primers[edit]

The 16S rRNA gene is used for phylogenetic studies as it is highly conserved between different species of bacteria and archaea. Carl Woesepioneered this use of 16S rRNA. It is suggested that 16S rRNA gene

can be used as a reliable molecular clock because 16S rRNA sequences from distantly related bacterial lineages are shown to have similar functionalities.[7] Some (hyper)thermophilic archaea (i.e. order Thermoproteales) contain 16S rRNA gene introns that are located in highly conserved regions and can impact the annealing of "universal" primers.[8] Mitochondrial and chloroplastic rRNA are also amplified.

The most common primer pair was devised by Weisburg *et al* and is currently referred to as 27F and 1492R; however, for some applications shorter amplicons may be necessary for example for 454 sequencing with Titanium chemistry (500-ish reads are ideal) the primer pair 27F-534R covering V1 to V3. Often 8F is used rather than 27F. The two primers are almost identical, but 27F has an M instead of a C. AGAGTTTGATC**M**TGGCTCAG compared with 8F.

Primer name	Sequence (5' - 3')	Reference
8F	AGA GTT TGA TCC TGG CTC AG	[11][12]
U1492R	GGT TAC CTT GTT ACG ACT T	same as above
928F	TAA AAC TYA AAK GAA TTG ACG GG	[13]
336R	ACT GCT GCS YCC CGT AGG AGT CT	as above

1100F	YAA CGA GCG CAA CCC	
1100R	GGG TTG CGC TCG TTG	

337F	GAC TCC TAC GGG AGG CWG CAG	
907R	CCG TCA ATT CCT TTR AGT TT	
785F	GGA TTA GAT ACC CTG GTA	
805R	GAC TAC CAG GGT ATC TAA TC	
533F	GTG CCA GCM GCC GCG GTA A	
518R	GTA TTA CCG CGG CTG CTG G	
27F	AGA GTT TGA TCM TGG CTC AG	[14]
1492R	CGG TTA CCT TGT TAC GAC TT	as above

PCR and NGS applications[edit]

In addition to highly conserved primer binding sites, 16S rRNA gene sequences contain hypervariable regions that can provide species-specific signature sequences useful for identification of bacteria As a

result, 16S rRNA gene sequencing has become prevalent in medical microbiologyas a rapid and cheap alternative to phenotypic methods of bacterial identification.[17] Although it was originally used to identify
bacteria, 16S sequencing was subsequently found to be capable of reclassifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been

successfully cultured. With Third-generation sequencing coming to many labs, simultaneous
identification of thousands of 16S rRNA sequences is possible within hours, allowing metagenomic studies, for example of the gut flora.

Hypervariable Regions[edit]

The bacterial 16S gene contains nine hypervariable regions (V1-V9) ranging from about 30-100 base pairs long that are involved in the secondary structure of the small ribosomal subunit.[23] The degree of conservation varies widely between hypervariable regions, with more conserved regions correlating to higher-level taxonomy and less conserved regions to lower levels, such as genus and species. While the entire 16S sequence allows for comparison of all hypervariable regions, at approximately 1500 base pairs long it can be prohibitively expensive for studies seeking to identify or characterize diverse bacterial communities. These studies commonly utilize the Illumina platform, which produces reads at rates 50-fold and 12,000-fold less expensive than 454 pyrosequencing and Sanger sequencing, respectively. While cheaper and allowing for deeper community coverage, Illumina sequencing only produces reads 75-150 base pairs long (250 to 300 base pairs with Illumina MiSeq), and has no established protocol for reliably assembling the full gene in community samples. Full hypervariable regions can be assembled from a single Illumina run, however, making them ideal targets for the platform.

While 16S hypervariable regions can vary dramatically between bacteria, the 16S gene as a whole maintains
greater length homogeneity than its Eukaryotic counterpart, which can make alignments easier. Additionally, the 16S gene contains highly conserved sequences between hypervariable regions, enabling the design of universal primers that can reliably produce the same sections of the 16S sequence across different taxa. Although no hypervariable region can accurately classify all bacteria from Domain to Species, some can reliably predict specific taxonomic levels. Many community studies select semi-conserved hypervariable regions like the V4 for this reason, as it can provide resolution at the phylum level as accurately as the full 16S gene. While lesser-conserved regions struggle to classify new species when higher order taxonomy is unknown, they are often used to detect the presence of specific pathogens. In one study by Chakravorty et al. in 2007, they characterized the V1-V8 regions of a variety of pathogens in order to determine which hypervariable regions would be most useful to include for disease-specific and broad assays. Amongst other findings, they noted that the V3 region was best at identifying the genus for all pathogens tested, and that V6 was the most accurate at differentiating species between all CDC-watched pathogens tested, including Anthrax.

While 16S hypervariable region analysis is a powerful tool for bacterial taxonomic studies, it struggles to differentiate between closely related species. In the families Enterobacteriaceae, Clostridiaceae, and

Peptostreptococcaceae, species can share up to 99% sequence similarity across the full 16S gene.[30] As a result, the V4 sequences can differ by only a few nucleotides, leaving reference databases unable to reliably classify these bacteria at lower taxonomic levels. By limiting 16S analysis to select hypervariable regions, these studies can fail to observe differences in closely related taxa and group them into single taxonomic units, therefore underestimating the total diversity of the sample. Furthermore, bacterial genomes can house multiple 16S genes, with the V1, V2, and V6 regions containing the greatest intraspecies diversity While not the most precise method of classifying bacterial species, analysis of the hypervariable regions remains one of the most useful tools available to bacterial community studies.

Genomics of extremophiles

Exploring natural genomic variability

Until recently, studies of extremophiles focused on physiological parameters as well as targeted and medium-throughput gene expression analysis. The development of rapid and cost-efficient next-generation sequencing technologies has cleared the way for studying both the molecular bases of these adaptations on a global scale and the evolutionary mechanisms driving them. In this context, comparisons of closely related extremophile and non -extremophile species are particularly valuable, because their genomes can be easily and reliably aligned, allowing genomic changes to be pinpointed at high resolution. The family of Brassicaceae is especially suited for this type of study, because of the development of *Arabidopsis thaliana* as a model organism, and because of the availability of a number of closely related extremophiles in possession of many of the characteristics that make *Arabidopsis* a good model (including a small genome, the possibility of genetic transformation, and a short life-cycle). The utility of Brassicaceae genomics in the study of stress tolerance was illustrated in 2011 by the publication of *Arabidopsis lyrata* [2] and *Thellungiella parvula* [3] genomes. Very recently, the sequencing of *Thellungiella salsuginea* [4] has complemented this set of resources. These three species have evolved to differ from *A. thaliana* in their stress tolerance within the last 7 to 12 million years (Figure (Figure1).1). Their study has focused on

structural changes as witnesses of forces driving the evolution of these genomes [2-4], as well as relating these structural changes to functions that may be related to stress tolerance.

Phylogenetic relationships between selected genome-sequenced Brassicaceae species relevant for the study of adaptation to extreme abiotic conditions. The phylogeny was constructed with sequences corresponding to the nuclear ribosomal ITS-1, 5.8S ribosomal RNA, and ITS-2 region identified in five species: *Arabidopsis thaliana* (U43225), *Arabidopsis lyrata* (DQ165338), *Thellungiella parvula* (Blast search on *T. parvula* transcriptome version 1.1, available at [7]), *Thellungiella salsuginea* (AF137564), and *Carica papaya* (AY461547), which serves as the outgroup. Alignments were performed with MAFFT, refined in BioEdit, and used for construction of the tree by neighbor joining in Mega 5. Mya, million years ago.

Structural changes and evolutionary forces

Structural genomic comparisons show that, despite significant differences in genome sizes, large regions of co-linearity are present between the four species [2,4,5]. Differences in genome size could be explained mainly by differences in the intergenic regions, where repeated sequences and transposable elements are commonly found. While in *A. thaliana* large numbers of microdeletions in these areas are suspected, resulting in a reduction of genome size [2], recent activity of transposable elements is thought to be one of the main reasons for the expansion of the *T. salsuginea* [4] and *A. lyrata* [2] genomes. The latter elements are also thought to be one of the factors at the origin of so-called taxonomically restricted, or orphan, genes and gene families, which are new genes that have recently arisen in a taxon. Wu *et al*. [4] show the *T. salsuginea* genome to contain 984 families of such genes, the functions of which still remain to be explored. Finally, tandem duplication, segmental duplication and retrotransposon-related gene duplication may act on genome structure, and also may be related to functional adaptation, both via modifying gene expression and by providing an opportunity for functional diversification. Retrotransposition as a source of gene duplication is found to be especially common in the extremophile *T. salsuginea*, relative to *A. thaliana* [4].

How genomic changes impact stress tolerance

To elucidate how and which structural genomic changes may influence the stress tolerance of extremophile organisms, both Dassanayake *et al*. [3] and Wu *et al*. [4] take advantage of *a priori* knowledge of gene function, as inferred through sequence homology, by applying this functional information to a focus on copy number variations. A simple method for identifying the functional categories whose constituent genes are subject to recent expansion is to perform gene set enrichment analyses. These analyses are frequently based on gene ontology annotations and can be used to identify functional groups of genes (or annotations) that are statistically over- or underrepresented in one genome compared with another. In both *Thellungiella* studies [3,4], gene set enrichment analysis shows that numerous categories of genes already known to be related to abiotic stress, including 'response to salt stress', 'abscisic acid stimulus', 'transporter activity' and 'development', are indeed overrepresented in the genomes of these halophytes compared with *A. thaliana*. This finding is of fundamental importance, as it demonstrates that positive selection for duplications of existing stress-response genes is likely to play a part in the adaptation to high abiotic stress environments.

Manual analysis and annotation of gene families that have undergone selective expansion in extremophiles is then used by Wu *et al*. in *T. salsuginea* to highlight specific genes and processes involved in stress tolerance, including genes encoding transcription factors, abscisic acid biosynthetic enzymes, a key enzyme involved in wax production, and the sodium transporter HKT1. The latter gene is of particular interest in *T. salsuginea* as *HKT1* has not only been triplicated with respect to *A. thaliana*, but one of the three copies has also undergone a significant increase in expression, most likely related to changes in the *cis*-regulatory region. Such changes have also been recorded for another sodium transporter encoded by the *salt overly sensitive 1* (*SOS1*) gene. For this gene, both *T. parvula* and *T. salsuginea* copies exhibit homologous promoter regions and high expression, whereas the *A. thaliana* transporter differs in promoter sequence and is expressed at lower levels , providing an example of how genomic changes in non-coding sequences may affect physiology.

Future challenges and directions

The recent work on *Thellungiella* [3,4] shows that, even for closely related species, successful adaptation to abiotic stress is likely to involve the combination of numerous genomic changes related to known but also to novel genes, which are driven by various evolutionary mechanisms. The studies provide a starting point for a fresh approach, namely the genome-wide, and thus holistic, study of the molecular bases of adaptation, and pave the way for the use of systems biology tools to construct and model metabolic and regulatory networks. These models can, in a next step, be expanded to combine other forms of high-throughput data, such as

metabolomic results and/or observations on small RNAs, DNA methylation and other forms of epigenetic regulation . At the same time, targeted approaches aiming to identify the function of individual genes are still needed, as even the best models will be incomplete until they are able to incorporate and understand the numerous orphan genes and gene families, which may well be the most innovative features of extremophile genomes. In the same vein, the most complete regulatory networks would remain hypothetical until backed up by experimental evidence.

We anticipate that the discussed studies, exploiting natural genetic variability of land plants to study the evolutionary processes of adaptation to extreme environments, will be inspirational for the development of similar approaches in other organisms. For instance, algae have also colonized environments spanning a wide range of abiotic factors. Meta-comparisons of the evolutionary mechanisms underlying adaptation across lineages and kingdoms will then provide insights into both conserved and specific mechanisms, and increase our understanding of the general principles underlying adaptation.

Random Amplified Polymorphic DNA (RAPD)

Introduction

Random Amplified Polymorphic DNA (RAPD)

markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence.

How It Works

Unlike traditional PCR analysis, RAPD (pronounced "rapid") does not require any specific knowledge of the DNA sequence of the target organism: the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence. For example, no fragment is produced if primers annealed too far apart or 3' ends of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel.

Example

RAPD is an inexpensive yet powerful typing method for many bacterial species.

Silver-stained polyacrylamide gel showing three distinct RAPD profiles generated by primer OPE15 for Haemophilus ducreyi isolates from Tanzania, Senegal, Thailand, Europe, and North America.

Selecting the right sequence for the primer is very important because different sequences will produce different band patterns and possibly allow for a more specific recognition of individual strains.

Limitations of RAPD

Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely.

PCR is an enzymatic reaction, therefore the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Thus, the RAPD technique is notoriously laboratory dependent and needs carefully developed laboratory protocols to be reproducible.

Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret.

Developing Locus-specific, Co-Dominant Markers from RAPDs

The polymorphic RAPD marker band is isolated from the gel.

It is amplified in the PCR reaction.

The PCR product is cloned and sequenced.

New longer and specific primers are designed for the DNA sequence, which is called the *Sequenced Characterized Amplified Region Marker (SCAR).*

Restriction fragment length polymorphism

In molecular biology, **restriction fragment length polymorphism** (**RFLP**) is a technique that exploits variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules from differing locations of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated. In **RFLP analysis**, the DNA sample is broken into pieces (digested) by restriction enzymes and the resulting *restriction fragments* are separated according to their lengths by gel electrophoresis. Although now largely

obsolete due to the rise of inexpensive DNA sequencing technologies, RFLP analysis was the first DNA profiling technique inexpensive enough to see widespread application. RFLP analysis was an important tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing.

Analysis (technology)

The basic technique for the detection of RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest. The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure.

Examples

There are two common mechanisms by which the size of a particular restriction fragment can vary. In the first schematic, a small segment of the genome is being detected by a DNA probe (thicker line). In allele "A", the genome is cleaved by a restriction enzyme at three nearby sites (triangles), but only the rightmost fragment will be detected by the probe. In allele "a", restriction site 2 has been lost by a mutation, so the probe now detects the larger fused fragment running from sites 1 to 3. The second diagram shows how this fragment size variation would look on a Southern blot, and how each allele (two per individual) might be inherited in members of a family.

In the third schematic, the probe and restriction enzyme are chosen to detect a region of the genome that includes a variable number tandem repeatsegment (boxes in schematic diagram). In allele "c" there are five repeats in the VNTR, and the probe detects a longer fragment between the two restriction sites. In allele "d" there are only two repeats in the VNTR, so the probe detects a shorter fragment between the same two restriction sites. Other genetic processes, such as insertions, deletions, translocations, and inversions, can also lead to RFLPs. RFLP tests require much bigger samples of DNA than do short tandem repeat (STR) tests.

Applications

Analysis of RFLP variation in genomes was a vital tool in genome mapping and genetic disease analysis. If researchers were trying to initially determine the chromosomal location of a particular disease gene, they would analyze the DNA of members of a family afflicted by the disease, and look for RFLP alleles that show a similar pattern of inheritance as that of the disease (see Genetic linkage). Once a disease gene was localized, RFLP analysis of other families could reveal who was at risk for the disease, or who was likely to be a carrier of the mutant genes.

RFLP analysis was also the basis for early methods of genetic fingerprinting, useful in the identification of samples retrieved from crime scenes, in the determination of paternity, and in the characterization of genetic diversity or breeding patterns in animal populations.

Alternatives

The technique for RFLP analysis is, however, slow and cumbersome. It requires a large amount of sample DNA, and the combined process of probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing, and autoradiography could take up to a month to complete. A limited version of the RFLP method that used oligonucleotide probes was reported in 1985. The results of the Human Genome Project have largely replaced the need for RFLP mapping, and the identification of many single-nucleotide polymorphisms (SNPs) in that project (as well as the direct identification of many disease genes and mutations) has replaced the need for RFLP disease linkage analysis (see SNP genotyping). The analysis of VNTR alleles continues, but is now usually performed by polymerase chain reaction (PCR) methods. For example, the standard protocols for DNA fingerprinting involve PCR analysis of panels of more than a dozen VNTRs.

RFLP is still a technique used in marker assisted selection. Terminal restriction fragment length polymorphism (TRFLP or sometimes T-RFLP) is a molecular biology technique initially developed for characterizing bacterial communities in mixed-species samples. The technique has also been applied to other groups including soil fungi.

TRFLP works by PCR amplification of DNA using primer pairs that have been labeled with fluorescent tags. The PCR products are then digested using RFLP enzymes and the resulting patterns visualized using a DNA sequencer. The results are analyzed either by simply counting and comparing bands or peaks in the TRFLP profile, or by matching bands from one or more TRFLP runs to a database of known species. The technique is similar in some aspects to DGGE or TGGE.

The sequence changes directly involved with an RFLP can also be analyzed more quickly by PCR. Amplification can be directed across the altered restriction site, and the products digested with the restriction enzyme. This method has been called Cleaved Amplified Polymorphic Sequence (CAPS). Alternatively, the amplified segment can be analyzed by Allele specific oligonucleotide (ASO) probes, a process that can often be done by a simple Dot blot.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc MB COURSE NAME: MARINE MICROBIOLOGY

COURSE CODE: 18MBP105A UNIT: II BATCH-2018-2020

Sl. No	QUESTIONS	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Particulate (organic) material which is only partly disintegrated is called as_____	demeris	divergent	detritus	debris	detritus
2	An organism which grows preferentially in high salinities.	Halophile	Barophile	Chemophile	Divergephile	Halophile
3	Living together of two organisms with protecting each other by producing chemicals is called as	Antagonism	Commensalism	Symbiosis	Mutualism	Antagonism
4	Which were the investigators lived at the same time?	Koch and Pasteur	Darwin and Woese	Van Leeuenhoek and Ricketts	Berg and Hooke	Koch and Pasteur
5	The unifying feature of the archaea that distinguishes them from the bacteria is	habitats which are extreme environments with regard to acidity	absence of a nuclear membrane temperature	presence of a cell wall containing a characteristic outer membrane	cytoplasmic ribosomes that are 70S	habitats which are extreme environments with regard to acidity
6	Which instrument is used for sterilization below 100° C	Flame	Autoclave	Filters	Desiccators	Flame
7	_____ is the last phase in growth curve	Log	Lag	stationary	death	death
8	RNA to DNA is called as	replication	biosynthesis	translation	reverse transcription	reverse transcription
9	The common word for bacteria which are curved in shape is	cocci	bacilli	spirilla	comma	comma
10	Single or clusters of flagella at both	monotrichous	peritrichous	amphitrichous	peritrichous	amphitrichous

	poles is known as					
11	Which of the following bacterial genera (that produces endospore) have medical importance?	E coli	Bacillus	Salmonella typhi	vibrio	Bacillus
12	Swimming towards a chemical of bacteria is termed as	positive chemotaxis	negative chemotaxis	phototaxis	magnetotaxis	positive chemotaxis
13	_____ group of bacteria grows in high pressure	Halophiles	Barophiles	thermophiles	psychrophiles	Barophiles
14	The group of gram positive bacteria having low G+C contents are called as	cyanobacteria	Nanobacteria	Firmicutes	Actinobacteria	Actinobacteria
15	Which of the following articles cannot be sterilized in an autoclave?	Gloves	Culture media	Dressing material	sugars	sugars
16	Which of the following disinfectants act by disrupting microbial membranes?	Cationic detergents	Halogens	Heavy metals	Aldehydes	Cationic detergents
17	Which of the following is best to sterilize heat labile solutions?	Dry heat	Autoclave	Membrane filtration	Pasteurization	Membrane filtration
18	All the following are considered eukaryotes except	archaea	fungi	protozoa	humans	archaea
19	_____are organism which grows at high pressure rather than at atmospheric pressure.	Barophile	halophile	thermophile	neutrophil	Barophile
20	Which cell type is considered to have the oldest ancestor?	Archaea	Bacteria	Eukarya	they all share the same ancestor	Archaea

21	Viable count method is used to count	only viable cells	only dead cells	Both a and b	None of the above	acetyl-CoA
22	The concept of putting microbes to help clean up the environment is called	pasteurization	bioremediation	fermentation	biolistics	bioremediation
23	Which of the following is not employed as an oxidation method?	Oxidation ponds	Trickling filters	Contact aerators	aeration ponds	Contact aerators
24	The filtering medium of trickling filters is coated with microbial flora, known as	zoological film	geological film	biofilm	physiological film	zoological film
25	Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate	water rich in organic carbon wastes but poor in phosphate	water those are anoxic	water rich in phosphate wastes but poor in organic carbon	water rich in organic matters	water rich in organic carbon wastes but poor in phosphate
26	The biogas production process takes place at the temperature	lesser than 25°C	25-40°C	45-60°C	45-70°C	45-60°C
27	What is an anaerobic digester?	New diet drink	Microbe that eats hazardous waste	Method to convert agricultural waste into a biogas	methanol production	Method to convert agricultural waste into a biogas
28	The use of microbes to break down synthetic waste products such as polychlorinated biphenyls is called	bioinformatics	biolistics	biotechnology	bioremediation	bioremediation
29	The use of microbes to break down synthetic waste products such as	bioinformatics	biolistics	biotechnology	bioremediation	bioremediation

	polychlorinated biphenyls is called Activated sludge contains large number of	bacteria	Yeasts and molds	protozoa	virus	bacteria
30	_____ group of bacteria grows in high temperature	Halophiles	Basophiles	thermophiles	psychrophiles	thermophiles
31	Bacteria which need oxygen for growth are called	Thermophilic bacteria	Microaerophilic bacteria	Facultative anaerobic bacteria	Anaerobic bacteria	Microaerophilic bacteria
32	pH required for the growth of bacteria is	6.8 – 7.2	5.6– 8.2	3.0 – 6.0	8.0– 14.0	6.8 – 7.2
33	In petridish count method, the colonies are expressed as	CFU	per ml	CFU/ml	All the above	R
34	The ion that is required in trace amounts for the growth of bacteria is	Calcium	Magnesium	Cobalt	Sodium	Cobalt
35	Expansion of RFLPs	Restriction Fragment Length Polymorphism	Restriction Filament Length polymorphism	Rapid Fragment Length Polymorphism	Rapid Filament Length Polymorphism	FADH2
36	Aerobic respiration differs from anaerobic respiration in which of the following respects?	Anaerobic respiration is glycolysis	Aerobic respiration requires the electron transport chain	The final electron acceptors are different	Aerobic respiration produces less ATP	Anaerobic respiration is glycolysis
37	Acid loving group of organism are called as	Acidophiles	alkalinophiles	barophiles	halophiles	Acidophiles

38	The group of gram positive bacteria having low G+C contents are called as	cyanobacteria	Nanobacteria	Firmicutes	Actinobacteria	Firmicutes
39	The predominant kind of bacteria causing spoilage in fish at chilling temperature is	species of *Pseudomonas*	*Micrococcus*	*Bacillus*	vibrio	species of *Pseudomonas*
40	Preservation of foods by using salts and sugars works by	raising pH	lowering osmotic pressure	creating a hypertonic environment	creating a hypotonic environment	raising pH
41	Removal of solids is generally considered as a	Primary treatment	Secondary treatment	Tertiary treatment	treatment	Primary treatment
42	All of the following species are considered coliforms except	Enterobacter aerogenes	Klebsiella pneumoniae	Salmonella typhi	Escherichia coli	Salmonella typhi
43	The sulfur pearl of Namibia, Thiomargarita namibiensis	is the world's largest bacterium	stores high concentrations of nitrate in a huge internal vacuole which takes up 98% of the organisms volume	micro bacteria	is the world's smallest bacterium	is the world's largest bacterium
44	Chemical precipitation of phosphorus is	primary treatment	secondary treatment	tertiary treatment	fourth treatment	secondary treatment
45	Which of the following is correct?	Ultramicrobacteria are nanobacteria	Ultramicrobacteria or nanobacteria are so numerous	ultrabacteria not bacteria	ultramicrobacteria are less	Ultramicrobacteria or nanobacteria are so numerous that

			that they are a major food source for heterotrophic flagellates			they are a major food source for heterotrophic flagellates
46	Coliforms are used as indicator organisms because	they are absent wherever enteric pathogens are present	a testing procedure with great specificity is easy to perform	no change	they present everywhere	a testing procedure with great specificity is easy to perform
47	Hyphomycete fungi	produce nonmotile tetaradiate conidia	produce motile tetaradiate conidia	no cilia no flagella	no true fungi	produce nonmotile tetaradiate conidia
48	Loss of carbon through the microbial loop in oligotrophic environments is _____ to/than the loss of carbon through the microbial loop in copiotrophic environments	greater	Lesser	approximately equal	half	approximately equal
49	Metabolism of dissolved organic material released by phytoplankton allows heterotrophic bacteria to become part of the particulate organic matter that is passed up the food web to be metabolized and released as mineral elements and CO2 at each transfer. This sentence describes the	microbial loop	Winogradsky column	Redfield ratio	Gibbs free energy	microbial loop
50	Which of the following is correct?	All members of	All members of	All members of	No member of	All members of

		Hyphomycetes are also members of Fungi, but not all members of Fungi are members of Hyphomycetes	Fungi are also members of Hyphomycetes, but not all members of Hyphomycetes are members of Fungi	Hyphomycetes are members of Fungi, and all members of Fungi are members of Hyphomycetes	Hyphomycetes is a member of Fungi	Hyphomycetes are also members of Fungi, but not all members of Fungi are members of Hyphomycetes
51	The amount of oxygen dissolved in hypolimnion water in the winter is _____ to/than the amount of oxygen dissolved in hypolimnion water in the summer	greater	Lesser	approximately equal to	half	approximately equal to
52	Members of all of the following genera of bacteria typically are found in a maturing Winogradsky column except	Clostridium	Rhodospirillum	Chlorobium	Escherichia	Rhodospirillum
53	Because it can be used with a variety of media and allow a resuscitation step the _____ technique has become the common and often preferred method of evaluating the microbiological characteristics of water.	most probable number	Winogradsky	MUG	membrane filtration	most probable number
54	Which of the following is correct?	A Winogradsky	A Winogradsky	no winogradsky	A Winogradsky	A Winogradsky

		column is used to filter out microorganisms from a water sample taken from a deep black smoker	column is used to demonstrate interactions and gradients that occur in aquatic environments		column is used to filter out microorganisms from non-aquatic environments	column is used to demonstrate interactions and gradients that occur in aquatic environments
55	Trickling filter comes under	primary treatment	secondary treatment	tertiary treatment	no treatment	secondary treatment
56	Trickling filter comes under Which of the following species in water reveal the presence of fecal pollution of human or animal origin?	primary treatment E.coli	secondary treatment Fecal Streptococci	tertiary treatment Clostridium perfringens	no treatment Salmonella	secondary treatment E.coli
57	Cytoplasmic inclusions exclude	ribosomes	mesosomes	fat globules	flagella	flagella
58	Activated sludge undergoes	Primary treatment	Secondary treatment	Tertiary treatment	fourth treatment	Secondary treatment
59	The rate of flux of oxygen in air is _____ to/than the rate of flux of oxygen in water.	greater	Lesser	approximately equal	half	greater
60	_____ is an environment like _____ where microorganisms are functioning in an extremely thin film of water and where oxygen-containing air is close to them.	low oxygen diffusion environment; soils	high oxygen diffusion environment; soils	low oxygen diffusion environment; lakes	high oxygen diffusion environment; lakes	high oxygen diffusion environment; soils

Unit III

Syllabus

Microbiology of degradation of xenobiotic environment: Ecological considerations, decay behaviour, degradative plasmids, hydrocarbons, oil pollution, surfactants, pesticides, Bioremediation:- Factors affecting bioremediation – role of microbes in the marine nutrient cycles – diseases of marine organisms and its impact on marine biodiversity. Marine Bioproducts.

Microbiology of degradation of xenobiotic environment:

Xenobiotics

It is derived from a greek word "Xenos" meaning 'foreign or strange'. Xenobiotics are those chemicals which are man-made and do not occur naturally in nature. They are usually synthesised for industrial or agricultural purposes eg. Aromatics, pesticides, hydrocarbons, plastics, lignin etc… they are also called RECALCITRANTS as they can resist degradation to maximum level.

BIODEGRADATION:

According to the definition by the International Union of Pure and Applied Chemistry, the term biodegradation is "Breakdown of a substance catalyzed by enzymes in vitro or in vivo. In other words, defined as the ability of microorganisms to convert toxic chemicLS (XENOBIOTICS) to simpler non-toxic compounds by synthesis of certain enzymes. Biodegradation of xenobiotics can be affected by substrate specificity, nutrition source, temperature, pH etc…

SOURCES OF XENOBIOTICS:

Petrochemical Industry: oil/gas industry, refineries. Produces basic chemicals eg., vinyl chloride and benzene.
Plastic Industry: closely related to the petrochemical industry. It uses a number of complex organic compounds such as antioxidants, plasticizers, cross-linking angents.
Pesticide Industry: Most commonly found. The structures aare benzene and its derivatives.
Paint Industry: major ingredient are solvents, xylene, toluene, methyl ethyl ketone, methyl.
Others: Electronic industry, textile industry, pulp and paper industry, cosmetics and pharmaceutical industry, wood preservation.

BIODEGRADATION OF PESTICIDES:

Pesticides are substances meant for destroying or mitigating any pest. They are a class od biocide. The most common use of pesticide is as plant protection products (also known as crop protection products). It includes: herbicide, insecticide, nemeticide, termicide, molluscicide, piscicide, avicide, rodenticide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant, and sanitizer.

DIFFERENT METHODS:

Detoxification: conversion of the pesticide molecule to a non-toxic compound. A single moiety in the side chain of a complex molecule is disturbed(removed), rendering the chemical non-toxic.

Degradation: Breakdown or transformation of a complex substrate into simpler products leading to mineralization. Eg., Thirum(fungicide) is degraded by a strain of *Pseudomonas* and the degradation products ar dimethylamine, proteins, sulpholipids, etc..

Conjugation(complex formation or addition reaction): an organism makes the substrate more complex or combines the pesticide with cell metabolites. Conjugation or the formation of addition product is accomplished by those organisms catalyzing the reaction of addition of an MINO cid, organic acid or methyl crown to the substrate thereby inactivating the pesticide.

Changing the spectrum of toxicity: some pesticides are designed to control one particular group of pests, butare metabolized to yield products inhibitory to entirely dissimilar groups of organisms, for eg., the fungicide PCNB is converted in soil to chlorinated benzoic acids tht kill plants.

There are many mechanisms involved on the biodegradation of pesticides and other contaminants. These may be summerised as follows:

Dehalogenation: nitrofen, DDT, cyanazine, propachlor.

Deamination: fluchloralin.

Decarboxylation: DDTc, biofenox, dichlorop-methyl

Methyl oxidation: bromacil

Hydroxylation: benthiocarb, bux insecticide.

BIODEGRADATION OF HYDROCARBONS:

A hydrocarbon is a organic compound consisting entirely of hydrogen and carbon. The majority of hydrocarbons found on earth naturally occur in crude oil. Aromatic hydrocarbons (arenes), alkanes, alkenes, cycloalkanes and alkyne-based compounds are different types of hydrocarbons.

BIODEGRADATION OF OIL POLLUTANTS:

Petroleum compounds are categorized into 2 groups

Aliphatic hydrocarbons eg., alkane, alcohol, aldehyde.
Aromatic hydrocarbon eg., benzene, phenol, toluene, catechol
Aromatic hydrocarbon are degraded aerobically and anaerobically.

Aerobic Degradation:

Aerobic Degradation are metabolized by a variety of bacteria, with ring fission accomplished by mono-and dioxygenases. Catechol and protocatechuate are the intermediates. Mostly found in aromatic compound degradation pathway.

Other Mechanisms:

Photometabolism in bacteria, this light-induced "bound oxygen" (OH) is used to oxidize substrates.
Under nitrate-reducing condition: Nitrate-reducing bacteria couple the oxidation of organic compound with water to the exergonic reduction of sulfate via sulfite to sulfide.

Some micoorganisms involved in the biodegradation of hydrcarbons

Organic pollutants	**Organisms**
Phenolic compound	*Achromobacter, Alcaligenes, Acinetobacter, Arthrobacter, azotobacter, Flavobacterium, Pseudomonas putida, Candida tropicalis, trichosporon cutaneoum, Aspergillus, Penicillium.*
Benzoate and related compound	*Arthrobacter, Bacillus spp., Micrococcus, P. putida*
Hydrocarbon	*E.coli, P. putida, P.aeruginosa, Candida*
Surfactants	*Alcaligenes, Achromobacter, Bacillus, Flavobacterium, Pseudomonas, Candida*
Pesticides	*P.aeruginosa – DDT, B.sphaericu – Linurin, Arthrobacter, P. cepacia – 2,4-D, P. cepacia – 2,4,5-T, Parathion.*

Genetic Regulation of Xenobiotic Degradation:

Plasmid Borne – mosrly in the genus *Pseudomonas,*

PLASMID	SUBSTRATE
TOL	Toluene, *m*-xylene, *p*-xylene
CAM	Camphor
NAH	Naphthalene
OCT	Octane, hexane, decane
pJP1	2,4-Dichlorophenoxy acetic acid
pAC25	3-Chlorobenzoate
SAL	Salicylat

POLYCYCLIC AROMATIC HYDROCARBON(PAH):

Bacteria, fungi, yeast, and algae have the ability to metabolize both lower and higher molecular weight PAHs found in the natural environment.

Most bacteria have been found to oxygenate the PAH initially to form dihydrodiol with a cis-configuration, which can be further oxidized to catechols.

Most fungi oxidise PAHs via a **cytochrome P** catalyzed **mono-oxygenase** reaction to form reactive arene oxides that can isomerize to phenols.

White-rot fungi oxidize PAHs via ligninases(lignin peroxidases and laccase) to form highly reactive quinones.

Bacterial strain degrading:

Compound	**Organisms**	**Metabolite**
Naphthalene	*Acinetobacter calcoaceticus,*	Naphthalene *cis* – 1,2- dihydrodio, 1,2-
	Alcaligenes denitrificans, Mycobacterium sp., Pseudomonas sp., Pseudomonas putida,	dihydroxynphthalene, 2-hydroxychromene-2- carboxylic acid, *trans* – o- hydroxybenzylidene pyruvic acid, salicylaldehyde, salicylic acid, catechol, gentisic acid, naphthalene *trans* – 1,2 - dihydrodiol
Acenaphthalene	*Beijerinckia sp., Pseudomonas putida, pseudomonas fluorescens, pseudomonas cepacia*	1- Acenaphthenol, 1- acenaphthenone, acenaphthene – cis – 1,2 – dihydrodiol, 1,2 – acenaphthenedione, 1,2 - dihydroxyacenaphthylene, 7,8 – diketonaphthyl -1- acetic acid, 3 – hydroxyphthalic acid.
Fluoranthene	*Alcaligenes denitrificana, Mycobacterium sp., Pseudomonas putida, Pseudomonas paucimobilis, Pseudomonas cepacia, Rhodococcus sp.,*	7 – acenaphthenone, 1 – acenaphthenone, 7 – hydroxyacenaphhenone, benzoic acid, phenyl acetic acid, adipic acid, 3 – hydroxymethyl – 4,5 – benzocoumarin, 9 – fluorenone – 1 – carboxylic acid, 8 – hydroxyl – 7 – methoxyfluoranthene, 9 – hydroxyfluorene, 9 – fluorenone, phthalic acid, 2 – carboxybenzaldehyde.
Pyrene	*Alcaligenes denitrificans, Mycobacterium sp., Rhodococcus sp.,*	Pyrene cis – and trans – 4,5 – dihydrodiol, 4 - hydroxyperinaphthenone, phthalic acid, 4 – phenanthroic acid, 1,2 – and 4,5 – dihydroxypyrene, cinnamic acid, cis – 2 – hydroxyl – 3 – (perinaphthenone – 9 – yl)

		propenic acid.
Chrysene	*Rhodococcus sp.,*	None determined
Benz(a) anthracene	*Alcaligenes denitrificans, Beijerinckia sp., Pseudomonas putida,*	Benz(a) anthracene cis - 1,2, cis – 8,9, and cis – 10,11 dihydrodiols, 1 – hydroxyl – 2 – anthranoic acid, 2 – hydroxyl – 3 – phenanthroic acid, 3 – hydroxyl – 2 – phenanthroic acid.
Benz(a) pyrene	*Beijerinckia sp., Mycobacterium sp.,*	Benz(a) pyrene cis – 7,8 – and cis 9,10 – dihydrodiols.

Xenobiotic

A **xenobiotic** is a foreign chemical substance found within an organism that is not normally naturally produced by or expected to be present within. It can also cover substances that are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal food.

Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators.

The term **xenobiotics**, however, is very often used in the context of pollutants such as dioxins and polychlorinated biphenyls and their effect on the biota, because xenobiotics are understood as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans. The term xenobiotic is derived from the Greek words ξένος (xenos) = foreigner, stranger and βίος (bios, vios) = life, plus the Greek suffix for adjectives -τικός, -ή, -ό (tic).

Xenobiotics may be grouped as carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides.

Xenobiotic metabolism

The body removes xenobiotics by xenobiotic metabolism. This consists of the deactivation and the excretion of xenobiotics, and happens mostly in the liver. Excretion routes are urine, feces, breath, and sweat. Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them (oxidation, reduction, hydrolysis and/or hydration of the xenobiotic), and then conjugating the active secondary metabolite with glucuronic acid, sulphuric acid, or glutathione, followed by excretion in bile or urine. An example of a group of enzymes involved in xenobiotic metabolism is hepatic microsomal

cytochrome P450. These enzymes that metabolize xenobiotics are very important for the pharmaceutical industry, because they are responsible for the breakdown of medications.

Organisms can also evolve to tolerate xenobiotics. An example is the co-evolution of the production of tetrodotoxin in the rough-skinned newt and the evolution of tetrodotoxin resistance in its predator, the Common Garter Snake. In this predator–prey pair, an evolutionary arms race has produced high levels of toxin in the newt and correspondingly high levels of resistance in the snake. This evolutionary response is based on the snake evolving modified forms of the ion channels that the toxin acts upon, so becoming resistant to its effects.

Xenobiotics in the environment

Xenobiotic substances are an issue for sewage treatment systems, since they are many in number, and each will present its own problems as to how to remove them (and whether it is worth trying to). It can be dangerous to the health.

Some xenobiotics are resistant to degradation. For example, they may be synthetic organochlorides such as plastics and pesticides, or naturally occurring organic chemicals such as polyaromatic hydrocarbons (PAHs) and some fractions of crude oil and coal. However, it is believed that microorganisms are capable of degrading almost all the different complex and resistant xenobiotics found on the earth Many xenobiotics produce a variety of biological effects, which is used when they are characterized using bioassay. Before they can be registered for sale in most countries, xenobiotic pesticides must undergo extensive evaluation for risk factors, such as toxicity to humans, ecotoxicity, or persistence in the environment. For example, during the registration process, the herbicide, cloransulam-methyl was found to degrade relatively quickly in soil.

Inter-species organ transplantation

The term **xenobiotic** is also used to refer to organs transplanted from one species to another. For example, some researchers hope that hearts and other organs could be transplanted from pigs to humans. Many people die every year whose lives could have been saved if a critical organ had been available for transplant. Kidneys are currently the most commonly transplanted organ. Xenobiotic organs would need to be developed in such a way that they would not be rejected by the immune system.

Biodegradation and Bioremediation

Biodegradation or biological degradation is the phenomenon of biological transformation of organic compounds by living organisms, particularly the microorganisms.

Biodegradation basically involves the conversion of complex organic molecules to simpler (and mostly non-toxic) ones. The term biotransformation is used for incomplete biodegradation of organic compounds involving one or a few reactions. Biotransformation is employed for the synthesis of commercially important products by microorganisms.

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. The microbes serve as scavengers in bioremediation. The removal of organic

wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used (by some authors) for bioremediation are bio-treatment, bio-reclamation and bio-restoration.

It is rather difficult to show any distinction between biodegradation and bioremediation. Further, in biotechnology, most of the reactions of biodegradation/bioremediation involve xenobiotic.

Xenobiotic:

Xenobiotic (xenos-foregin) broadly refer to the unnatural, foreign and synthetic chemicals such as pesticides, herbicides, refrigerants, solvents and other organic compounds. Microbial degradation of xenobiotic assumes significance, since it provides an effective and economic means of disposing of toxic chemicals, particularly the environmental pollutants.

Pseudomonas — The Predominant Microorganism For Bioremediation:

Members of the genus Pseudomonas (a soil microorganism) are the most predominant microorganisms that degrade xenobiotic. Different strains of Pseudomonas, that are capable of detoxifying more than 100 organic compounds, have been identified. The examples of organic compounds are several hydrocarbons, phenols, organophosphates, polychlorinated biphenyls (PCBs) and polycylic aromatics and naphthalene.

About 40-50 microbial strains of micro¬organisms, capable of degrading xenobiotics have been isolated. Besides Pseudomonas, other good examples are Mycobacterium, Alcaligenes, and Nocardia. A selected list of microorganisms and the xenobiotics degraded is given in Table 59.1.

TABLE 59.1 A selected list of microorganisms and the pollutants (xenobiotics) that are degraded by bioremediation

Microorganism	*Pollutant chemicals*
Pseudomonas sp	Aliphatic and aromatic hydrocarbons—alkylaminoxides, alkylammonium benzene, naphthalene, anthracene xylene, toluene, polychlorinated biphenyls (PCBs), malathion, parathion, organophosphates.
Mycobacterium sp	Benzene, branched hydrocarbons, cycloparaffins
Alcaligenes sp	Polychlorinated biphenyls, alkyl benzene, halogenated hydrocarbons.
Nocardia sp	Naphthalene, alkylbenzenes, phenoxyacetate.
Arthrobacter sp	Benzene, polycyclic aromatics, phenoxyacetate, pentachlorophenol.
Corynebacterium sp	Halogenated hydrocarbons, phenoxyacetate.
Bacillus sp	Long chain alkanes, phenylurea.
Candida sp	Polychlorinated biphenyls
Aspergillus sp	Phenols
Xanthomonas sp	Polycyclic hydrocarbons
Streptomyces sp	Halogenated hydrocarbons, phenoxyacetate.
Fusarium sp	Propanil
Cunninghamella sp	Polycyclic aromatics, polychlorinated biphenyls.

Consortia of microorganisms for biodegradation:

A particular strain of microorganism may degrade one or more compounds. Sometimes, for the degradation of a single compound, the synergetic action of a few microorganisms (i.e. a consortium or cocktail of microbes) may be more efficient. For instance, the insecticide parathion is more efficiently degraded by the combined action of *Pseudomonas aeruginosa* and *Pseudomonas stulzeri.*

Co-metabolism in biodegradation:

In general, the metabolism (breakdown) of xenobiotics is not associated with any advantage to the microorganism. That is the pollutant chemical cannot serve as a source of carbon or energy for the organism. The term co-metabolism is often used to indicate the non-beneficial (to the microorganism) biochemical pathways concerned with the biodegradation of xenobiotics. However, co- metabolism depends on the presence of a suitable substrate for the microorganism. Such compounds are referred to co-substrates.

Factors Affecting Biodegradation:

Several factors influence biodegradation. These include the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and O2 supply, temperature, pH and redox potential. Among these, the chemical nature of the substrate that has to be degraded is very important.

Some of the relevant features are given hereunder:

i. In general, aliphatic compounds are more easily degraded than aromatic ones.

Presence of cyclic ring structures and length chains or branches decrease the efficiency of biodegradation.

Water soluble compounds are more easily degraded.

Molecular orientation of aromatic compounds influences biodegradation i.e. ortho > para > meta.

The presence of halogens (in aromatic compounds) inhibits biodegradation.

Besides the factors listed above, there are two recent developments to enhance the biodegradation by microorganisms.

Bio-stimulation:

This is a process by which the microbial activity can be enhanced by increased supply of nutrients or by addition of certain stimulating agents (electron acceptors, surfactants).

Bio-augmentation:

It is possible to increase biodegradation through manipulation of genes. More details on this genetic manipulation i.e. genetically engineered microorganisms (GEMs), are described later. Bio-augmentation can also be achieved by employing a consortium of micro¬organisms.

Enzyme Systems for Biodegradation:

Several enzyme systems (with independent enzymes that work together) are in existence in the microorganisms for the degradation of xenobiotics. The genes coding for the enzymes of bio-degradative pathways may be present in the chromosomal DNA or more frequently on the plasmids. In certain microorganisms, the genes of both chromosome and plasmid contribute for the enzymes of biodegradation. The microorganism Pseudomonas occupies a special place in biodegradation.

selected list of xenobiotics and the plasmids containing the genes for their degradation is given in Table 59.2.

TABLE 59.2 A selected list of xenobiotics and the plasmids containing genes (in *Plasmodium*) for biodegradation

Xenobiotic	*Name of plasmid in* Pseudomonas
Naphthalene	NAH
Xylene	XYL
Xylene and toluene	TOL, pWWO, XYL-K
Salicylate	SAL
Camphor	CAM
3-Chlorobenzene	pAC25

Recalcitrant Xenobiotics:

There are certain compounds that do not easily undergo biodegradation and therefore persist in the environment for a long period (sometimes in years). They are labeled as recalcitrant.

There may be several reasons for the resistance of xenobiotics to microbial degradation:

i. They may chemically and biologically inert (highly stable).

Lack of enzyme system in the microorganisms for biodegradation.

They cannot enter the microorganisms being large molecules or lack of transport systems.

The compounds may be highly toxic or result in the formation highly toxic products that kill microorganisms.

There are a large number of racalcitrant xenobiotic compounds e.g. chloroform, freons, insecticides (DDT, lindane), herbicides (dalapon) and synthetic polymers (plastics e.g. polystyrene, polyethylene, polyvinyl chlorine).

It takes about 4-5 years for the degradation of DDT (75-100%) in the soil. A group of microorganisms (Aspergillus flavus, Mucor aternans, Fusarium oxysporum and Trichoderma viride) are associated with the slow biodegradation of DDT.

Bio-magnification:

The phenomenon of progressive increase in the concentration of a xenobiotic compound, as the substance is passed through the food chain is referred to as bio-magnification or bioaccumulation. For instance, the insecticide DDT is absorbed repeatedly by plants and microorganism.

When they are eaten by fish and birds, this pesticide being recalcitrant, accumulates, and enters the food chain. Thus, DDT may find its entry into various animals, including man. DDT affects the nervous systems, and it has been banned in some countries.

Types of Bioremediation:

The most important aspect of environmental biotechnology is the effective management of hazardous and toxic pollutants (xenobiotics) by bioremediation. The environmental clean-up process through bioremediation can be achieved in two ways—in situ and ex situ bioremediation.

In Situ Bioremediation:

In situ bioremediation involves a direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water). Addition of adequate quantities of nutrients at the sites promotes microbial growth. When these microorganisms are exposed to xenobiotics (pollutants), they develop metabolic ability to degrade them.

The growth of the microorganisms and their ability to bring out biodegradation are dependent on the supply of essential nutrients (nitrogen, phosphorus etc.). In situ bioremediation has been successfully applied for clean-up of oil spillages, beaches etc. There are two types of in situ bioremediation-intrinsic and engineered.

Intrinsic bioremediation:

The inherent metabolic ability of the microorganisms to degrade certain pollutants is the intrinsic bioremediation. In fact, the microorganisms can be tested in the laboratory for their natural capability of biodegradation and appropriately utilized.

Engineered in situ bioremediation:

The inherent ability of the microorganisms for bioremediation is generally slow and limited. However, by using suitable physicochemical means (good nutrient and O2supply, addition of electron acceptors, optimal temperature), the bioremediation process can be engineered for more efficient degradation of pollutants.

Advantages of in situ bioremediation:

Cost-effective, with minimal exposure to public or site personnel.

Sites of bioremediation remain minimally disrupted.

Disadvantages of in situ bioremediation:

Very time consuming process.

Sites are directly exposed to environmental factors (temperature, O2 supply etc.).

Microbial degrading ability varies seasonally.

Ex Situ Bioremediation:

The waste or toxic materials can be collected from the polluted sites and the bioremediation with the requisite microorganisms (frequently a consortium of organisms) can be carried out at designed places. This process is certainly an improvement over in situ bioremediation, and has been successfully used at some places.

Advantages of ex situ bioremediation:

Better controlled and more efficient process.

Process can be improved by enrichment with desired microorganisms.

Time required in short.

Disadvantages of ex situ bioremediation:

Very costly process.

Sites of pollution are highly disturbed.

There may be disposal problem after the process is complete.

Metabolic Effects of Microorganisms on Xenobiotics:

Although it is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, it is not always possible. This is evident from the different types of metabolic effects as shown below.

Detoxification:

This process involves the microbial conversion of toxic compound to a non¬toxic one. Biodegradation involving detoxification is highly advantageous to the environment and population.

Activation:

Certain xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous.

Degradation:

The complex compounds are degraded to simpler products which are generally harmless.

Conjugation:

The process of conjugation may involve the conversion of xenobiotics to more complex compounds. This is however, not very common.

Types of Reactions in Bioremediation:

Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

Aerobic bioremediation:

Aerobic biodegradation involves the utilization of O2 for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms. Two types of enzymes namely mono-oxygenases and- di-oxygenases are involved in aerobic biodegradation. Mono-oxygenases can act on both aliphatic and aromatic compounds while di-oxygenases oxidize aliphatic compounds.

Anaerobic bioremediation:

Anaerobic biodegradation does not require O2 supply. The growth of anaerobic microorganisms (mostly found in solids and sediments), and consequently the degradation processes are slow. However, anaerobic biodegradation is cost- effective, since the need for continuous O2 supply is not there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below.

Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dehaiogenation — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. The term de-chlorination is frequently used for dehaiogenation of chlorinated compounds.

Carboxylation and decarboxylation — toluene, cresol and benzoate.

Sequential Bioremediation:

In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

Biodegradation of Hydrocarbons:

Hydrocarbon are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of microorganisms e.g. *Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium* and *Nocardia.*

Biodegradation of Aliphatic Hydrocarbons:

The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are reclacitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride).

Biodegradation of Aromatic Hydrocarbons:

Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas.

The biodegradation of aromatic compounds basically involves the following sequence of reactions:

Removal of the side chains.

Opening of the benzene ring.

Most of the non-halogenated aromatic compounds undergo a series of reactions to produce catechol or protocatechuate. The bioremediation of toluene, L-mandelate, benzoate, benzene, phenol, anthracene, naphthalene, phenanthrene and salicylate to produce catechol is shown in Fig. 59.1. Likewise, Fig. 59.2, depicts the bioremediation of quinate, p-hydroxymandelate, p-hydroxybenzoyl formate, p-toluate, benzoate and vanillate to produce protocatechuate. Catechol and protocatechuate can undergo oxidative cleavage pathways.

Dehalogenation (i.e. removal of a halogen substituent from an organic compound) of halogenated compounds is an essential step for their detoxification. Dehalogenation is frequently catalysed by the enzyme di-oxygenase. In this reaction, there is a replacement of halogen on benzene with a hydroxyl group.

Most of the halogenated compounds are also converted to catechol and protocatechuate which can be metabolised (Fig. 59.4). Besides *Pseudomonas,* other microorganisms such as *Azotobacter, Bacillus* and *E. coli* are also involved in the microbial degradation of halogenated aromatic compounds.

Biodegradation of Polychlorinated Biphenyls (PCBs):

The aromatic chlorinated compounds possessing biphenyl ring (substituted with chlorine) are the PCBs e.g. pentachlorobiphenyl. PCBs are commercially synthesized, as they are useful for various purposes — as pesticides, in electrical conductivity (in transformers), in paints and adhesives. They are inert, very stable and resistant to corrosion

However, PCBs have been implicated in cancer, damage to various organs and impaired reproductive function. Their commercial use has been restricted in recent years, and are now used mostly in electrical transformers. PCBs accumulate in soil sediments due to hydrophobic nature and high bioaccumulation potential. Although they are resistant to biodegradation, some methods have been recently developed for anaerobic and aerobic oxidation by employing a consortium of microorganisms. Pseudomonas, Alkali genes, Corynebacterium and Acinetobacter. For more efficient degradation of PCBs, the microorganisms are grown on biphenyls, so that the enzymes of biodegradation of PCBs are induced.

Biodegradation of Some Other Important Compounds:

Organo-nitro Compounds:

Some of the toxic organo-nitro compounds can be degraded by microorganisms for their detoxification.

2, 4, 6-Trinitrotoluene (TNT):

Certain bacterial and fungal species belonging to Pseudomonas and Clostrium can detoxify TNT.

Nitrocellulose:

Hydrolysis, followed by anaerobic nitrification by certain bacteria, degrades nitrocellulose.

Synthetic detergents:

They contain some surfactants (surface active agents) which are not readily biodegradable. Certain bacterial plasmid can degrade surfactants.

Genetic Engineering for More Efficient Bioremediation:

Although several microorganisms that can degrade a large number of xenobiotics have been identified, there are many limitations in bioremediation:

i. Microbial degradation of organic compounds is a very slow process.

No single microorganism can degrade all the xenobiotics present in the environmental pollution.

Certain xenobiotics get adsorbed on to the particulate matter of soil and become unavailable for microbial degradation.

It is never possible to address all the above limitations and carry out an ideal process of bioremediation. Some attempts have been made in recent years to create genetically engineered microorganisms (CEMs) to enhance bio¬remediation, besides degrading xenobiotics which are highly resistant (recalcitrant) for breakdown. Some of these aspects are briefly described.

Genetic Manipulation by Transfer of Plasmids:

The majority of the genes responsible for the synthesis of bio-degradative enzymes are located on the plasmids. It is therefore logical to think of genetic manipulations of plasmids. New strains of bacteria can be created by transfer of plasmids (by conjugation) carrying genes for different degradative pathways.

If the two plasmids contain homologous regions of DNA, recombination occurs between them, resulting in the formation of a larger fused plasmid (with the combined functions of both plasmids). In case of plasmids which do not possess homologous regions of DNA, they can coexist in the bacterium (to which plasmid transfer was done).

The first successful development of a new strain of bacterium (Pseudomonas) by manipulations of plasmid transfer was done by Chakrabarty and his co-workers in 1970s. They used different plasmids and constructed a new bacterium called as superbug that can degrade a number of hydrocarbons of petroleum simultaneously.

United States granted patent to this superbug in 1981 (as per the directive of American Supreme Court). Thus, superbug became the first genetically engineered microorganism to be patented. Superbug has

played a significant role in the development of biotechnology industry, although it has not been used for large scale degradation of oil spills.

Creation of Superbug by Transfer of Plasmids:

Superbug is a bacterial strain of Pseudomonas that can degrade camphor, octane, xylene and naphthalene. Its creation is depicted in Fig. 59.5.

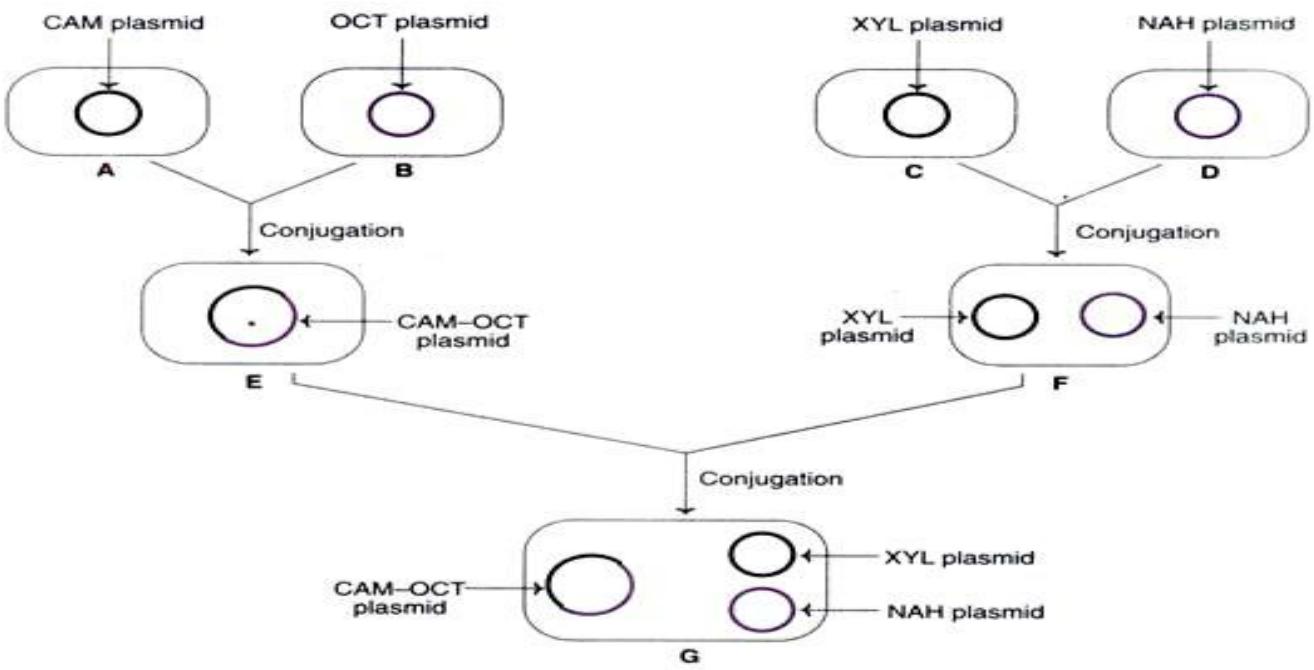


Fig. 59.5 : Creation of the superbug by transfer of plasmids (A, B, C, D, E, F and G are the different strains of bacteria containing the plasmids shown. Strain G is the superbug.)

The bacterium containing CAM (camphor- degrading) plasmid was conjugated with another bacterium with OCT (octane-degrading) plasmid. These plasmids are not compatible and therefore, cannot coexist in the same bacterium. However, due to the presence of homologous regions of DNA, recombination occurs between these two plasmids resulting in a single CAM-OCT plasmid. This new bacterium possesses the degradative genes for both camphor and octane.

Another bacterium with XYL (xylene-degrading) plasmid is conjugated with NAH (naphthalene-degrading) plasmid containing bacterium. XYL and NAH plasmids are compatible and therefore can coexist in the same bacterium. This newly, produced bacterium contains genes for the degradation of xylene and naphthalene.

The next and final step is the conjugation of bacterium containing CAM-OCT plasmid with the other bacterium containing XYL and NAH plasmids. The newly created strain is the superbug that carries

CAM-OCT plasmid (to degrade camphor and octane), XYL (xylene-degrading) plasmid and NAH (naphthalene-degrading) plasmid.

Development of Salicylate—Toluene Degrading Bacteria by Plasmid Transfer:

Some attempts have been made for the creation of a new strain of the bacterium Pseudomonas putida to simultaneously degrade toluene and salicylate. Toluene-degrading (TOL) plasmid was transferred by conjugation to another bacterium that is capable of degrading salicylate (due to the presence of SAL plasmid).

The newly developed strain of Pseudomonas can simultaneously degrade both toluene and salicylate. And this occurs even at a low temperature (0-5°C). However, the new bacterium is not in regular use, as more research is being conducted on its merits and demerits.

Genetic Manipulation by Gene Alteration:

Work is in progress to manipulate the genes for more efficient biodegradation. The plasmid pWWO of Pseudomonas codes for 12 different enzymes responsible for the meta-cleavage pathway (for the conversion of catechol and protocatechuate to pyruvate and acetaldehyde, for degradation of certain aromatic compounds. Some success has been reported to alter the genes of plasmid pWWO for more efficient degradation of toluene and xylene.

Genetically Engineered Microorganisms (GEMs) in Bioremediation:

Superbug is the first genetically engineered microorganism. Several workers world over have been working for the creation of GEMs, specifically designed for the detoxification of xenobiotics. A selected list of GEMs with a potential for the degradation of xenobiotics is given in Table 59.3. Almost all these CFMs have been created by transferring plasmids.

TABLE 59.3 A selected list of genetically engineered microorganisms (GEMs) with the potential xenobiotics that can be degraded

Genetically engineered microorganism (GEMs)	*Xenobiotic*
Pseudomonas diminuta	Parathion
P. oleovorans	Alkane
P. cepacia	2, 4, 5-Trichlorophenol
P. putida	Mono- and dichloro-aromatic compounds
Alcaligenes sp	2, 4-Dichlorophenoxy acetic acid
Acinetobacter sp	4-Chlorobenzene

Bio-surfactant Producing GEM:

A genetically engineered Pseudomonas aeruginosa has been created (by Chakarabarty and his group). This new strain can produce a glycolipid emulsifier (a bio-surfactant) which can reduce the surface tension of an oil water interface. The reduced interfacial tension promotes biodegradation of oils.

GEM for Degradation of Vanillate and SDS:

A new strain of Pseudomonas sp (strain ATCC 1915) has been developed for the degradation of vanillate (waste product from paper industry) and sodium dodecyl sulfate (SDS, a compound used in detergents).

GEMs and Environmental Safety:

The genetically engineered microorganisms (GEMs) have now become handy tools of biotechnologists. The risks and health hazards associated with the use of GEMs are highly controversial and debatable issues. The fear of the biotechnologists and even the general public is that the new organism (GEM), once it enters the environment, may disturb the ecological balance and cause harm to the habitat. Some of the GEMs may turn virulant and become genetic bombs, causing great harm to humankind.

Because of the risks involved in the use of GEMs, so far no GEM has been allowed to enter the environmental fields. Thus, the use of GEMs has been confined to the laboratories, and fully controlled processes of biodegradation

(usually employing bioreactors). Further, several pre-cautionary measures are taken while creating GEMs, so that the risks associated with their use are minimal.

Some researchers are of the opinion that GEMs will create biotechnological wonders for the environmental management of xenobiotics, in the next few decades. This may be possible only if the associated risks of each GEM is thoroughly evaluated, and fully assured of its biosafety.

Bioremediation of Contaminated Soils and Waste Lands:

to industrialization and extensive use of insecticides, herbicides and pesticides, the solids and waste lands world over are getting polluted. The most common pollutants are hydrocarbons, chlorinated solvents, polychlorobiphenyls and metals.

Bioremediation of soils and waste lands by the use of microorganisms is gaining importance in the recent years. In fact, some success has been reported for the detoxification of certain pollutants (e.g.

hydrocarbons) in the soil by microorganisms. Bioremediation of soils can be done by involving two principles-bio-stimulation and bio-augmentation.

Bio-stimulation in Soil Bioremediation:

Bio-stimulation basically involves the stimulation of microorganisms already present in the soil, by various means.

This can be done by many ways:

i. Addition of nutrients such as nitrogen and phosphorus.

Supplementation with co-substrates e.g. methane added to degrade trichloroethylene.

Addition of surfactants to disperse the hydrophobic compounds in water.

Addition of nutrient and co-substrates promote microbial growth while surfactants expose the hydrophobic molecules. In all these situations, the result is that there occurs bio-stimulation by effective bioremediation of polluted soil or waste land

Bio-augmentation in Soil Bioremediation:

Addition of specific microorganisms to the polluted soil constitutes bio-augmentation. The pollutants are very complex molecules and the native soil microorganisms alone may not be capable of degrading them effectively. The examples of such pollutants include polychlorobiphenyls (PCBs), trinitrotoluene (TNT), polyaromatic hydrocarbons (PAHs) and certain pesticides.

Based on the research findings at the laboratory level (with regard to biodegradation), it is now possible to add a combination of microorganisms referred to as consortium or cocktail of microorganisms, to achieve bio-augmentation. With the development of genetically engineered microorganisms (GEMs), they can be also used to bio-augment soils for very efficient bioremediation. But the direct use of GEMs in the soils is associated with several risks and health hazards.

Techniques of Soil Bioremediation:

The most commonly used methods for the bioremediation are soils are in situ bioremediation, land farming and slurry phase bioreactors.

In Situ Bioremediation of Soils:

In situ bioremediation broadly involves the biological clean-up of soils without excavation. This technique is used for the bioremediation of sub-surfaces of soils, buildings and road ways that are polluted. Sometimes, water (oxygenated) is cycled through the sub-surfaces for increasing the efficiency

of microbial degradation. There are two types of in situ soil bioremediation techniques- bioventing and phytoremediation.

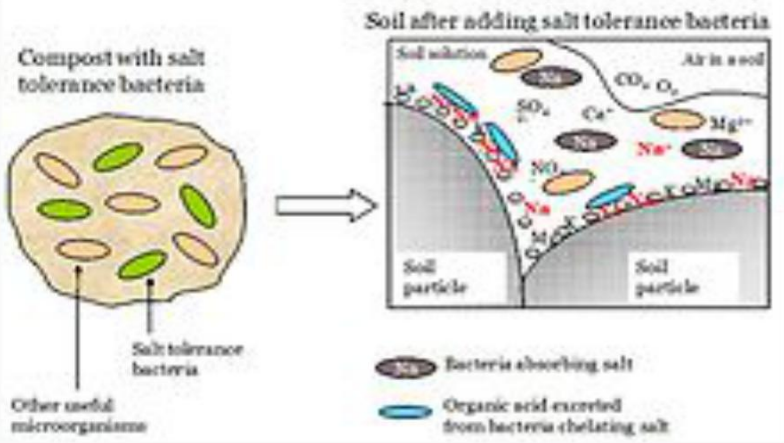
Bioventing:

This is very efficient and cost- effective technique for the bioremediation of petroleum contaminated soils. Bioventing involves aerobic biodegradation of pollutants by circulating air through sub-surfaces of soil. Although, it takes some years, bioventing can be used for the degradation of soluble paraffin's and polyaromatic hydrocarbons. The major limitation of this technique is air circulation which is not always practicable.

Phytoremediation:

Bioremediation by use of plants constitutes phytoremediation. Specific plants are cultivated at the sites of polluted soil. These plants are capable of stimulating the biodegradation of pollutants in the soil adjacent to roots (rhizosphere) although phytoremediation is a cheap and environmental friendly clean-up process for the biodegradation of soil pollutants, it takes several years.

Bioremediation



Mechanism of salt removal from tsunami affected soil by bioremediation

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from acontaminated site.[1] According to the United States EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances". Technologies can be generally classified as *in situ* or *ex situ*. *In situ*bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some

examples of bioremediation related technologies are phytoremediation, bioventing, bioleaching, landfarming,bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc.,that help in enhancing the growth of the pollution-eating microbes within the medium (biostimulation). For example, the US Army Corps of Engineers demonstrated that windrowing and aeration of petroleum-contaminated soils enhanced bioremediation using the technique of landfarming.[2] Depleted soil nitrogen status may encourage biodegradation of some nitrogenous organic chemicals, and soil materials with a high capacity to adsorb pollutants may slow down biodegradation owing to limited bioavailability of the chemicals to microbes.[4] Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as **bioremediators**.

However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil. Bone char has been shown to bioremediate small amounts of cadmium, copper, and zinc. A recent experiment, suggests that the removals of pollutants (nitrate, silicate, chromium and sulphide) from tannery wastewater were studied in batch experiments using marine microalgae. The assimilation of metals such as mercury into the food chain may worsen matters.Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. Some damaged artifacts at museums contain microbes which could be specified as bio remediating agents. In contrast to this situation, other contaminants, such as aromatic hydrocarbons as are common in petroleum, are relatively simple targets for microbial degradation, and some soils may even have some capacity to autoremediate, as it were, owing to the presence of autochthonous microbial communities capable of degrading these compounds.

The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: IMSc MB COURSE NAME: MARINE MICROBIOLOGY

COURSE CODE: 18MBP105A UNIT: III BATCH-2018-2020

Sl. No	QUESTIONS	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	The magnitude of BOD of wastewater is related to	bacterial count	amount of organic material	amount of inorganic material	viral count	amount of organic material
2	Biogas production is	a temperature-dependent process	a temperature independent process	an oxygen dependent process	none of the above	an oxygen dependen process
3	Iron bacteria can produce	slime	undesirable odors and tastes	no taste	extreme acidity	undesirable odors and tastes
4	Biomass	provides the U.S. with about 50% of its energy	consists largely of wood, animal, and human waste	is unlikely to be a major source of energy globally	offers the consumer high quality energy with low environmental impact	offers the consumer high quality energy with low environmental impact
5	Which is not a form of biomass energy?	Incineration of solid waste	Composting to produce methane	Ethanol and methanol production for auto fuel	Photovoltaic production of hydrogen	Incineration of solid waste
6	Which of the following statement is not correct?	The use of 25-40°C temperatures allows the biogas production to be more stable	The use of 25-40°C temperatures does not destroy potentially	The use of 25-40°C temperatures destroys potentially	None of the above	The use of 25-40°C temperatures does not destroy potentially harmful bacteria

			harmful bacteria	harmful bacteria		
7	Which of the following is not the biofertilisers producing bacteria?	Nostoc	Anabaena	volvox	Clostridium	Clostridium
8	Which of the following is capable of oxidizing sulfur to sulfates?	Thiobacillus thiooxidans	Desulfotomaculum	Rhodospirillum	Rhodomicrobium	Thiobacillus thiooxidans
9	The diagnostic enzyme for denitrification is	nitrate reductase	nitrate oxidase	nitro oxidoreductase	nitrate dehydrogenase	nitro oxidoreductase
10	The groups of symbiotic bacteria, which have the ability to fix nitrogen	derive their food and minerals from the legume, and in turn they supply the legume with some or all of its nitrogen	grow together for a mutual benefit is called symbiosis and so these bacteria are called symbiotic nitrogen-fixing bacteria	these bacteria are from the genus, Rhizobium	no change	derive their food and minerals from the legume, and in turn they supply the legume with some or all of its nitrogen
11	An example of a symbiotic nitrogen fixer is	Azotobacter	Beijerinckia	Clostridium	Rhizobium	Azotobacter
12	Which of the following statement is not true about composition of biogas?	It is composed almost exclusively of methane and carbon dioxide	It also contains with traces of H2S, N2, H2and CO	It also contains with traces of O2 and Cl2	no O2 and NO Co2	It also contains with traces of O2 and Cl2
13	The groups of bacteria which have the ability to fix nitrogen from air to soil are	symbiotic	Nonsymbiotic	both (a) and (b)	non symbiotic	symbiotic
14	Which are the main source of	Cyanobacteria	Bacillus	Streptococcus	coliform	Cyanobacteria

	biofertilisers?					
15	Degree of compost maturity can be assesed by	infrared technique	germination test	both (a) and (b)	no test	germination test
16	The diagnostic enzyme for nitrogen-fixing organisms is	nitrogenase	nitrate reductase	nitrate oxidase	no enzyme	nitrate reductase
17	Syntrophism involves	exchange of nutrients between two species	exchange of nutrients among species	no exchange of nutrients between two species	no exchange of nutrients among species	exchange of nutrients among species
18	Assimilative denitrification is done by	plants	Fungi	prokaryotes	virus	prokaryotes
19	Which of the following bacteria is associated with food poisoning due to consumption of sea fish?	Vibrio parahaemolyticus	V alginolyticus	V vulnificus	V. chlorae	Vibrio parahaemolyticus
20	Which of the following conditions can be caused by Plesiomonas?	Septicaemia	Gastroenteritis	Cellulites	edema	Septicaemia
21	Which of the following does not cause wound infection following exposure to sea water or infected shellfish?	Vibrio vulnificus	V. alginolyticus	V. cholerae	Aeromonas	V. cholerae
22	The DNA coding for the production of cholera toxin in Vibrio cholerae is on the	phage	Plasmid	chromosome	transposon	plasmid
23	Which of the following toxin resembles cholera toxin?	Stable toxin of E. coli	Diphtheria toxin	Labile toxin of Escherichia coli	Tetanus toxin	Stable toxin of E. coli

24	Which of the following bacteria lack a cell wall and are therefore resistant to penicillin?	Cyanobacteria	Mycoplasmas	Bdellovibrios	Spirochetes	Mycoplasmas
25	A cluster of polar flagella is called	lophotrichous	Amphitrichous	monotrichous	peritrichous	lophotrichous
26	The protein from which hook and filaments of flagella are composed of, is	keratin	Flagellin	gelatin	casein	flagellin
27	The cocci which mostly occur in single or pairs are	Streptococci	Diplococci	Tetracocci	None of these	Diplococci
28	Which of the following may contain fimbriae?	Gram-positive bacteria	Gram-negative bacteria	Both (a) and (b)	None of these	Gram-positive bacteria
29	Peptidoglycan accounts for _____ of the dry weight of cell wall in many gram positive bacteria	50% or more	About 10%	11%+ 0.22%	About 20%	50% or more
30	Bacteria having no flagella are unable to	move	Reproduces	stick to tissue surfaces	grow in nutrient agar	move
31	Which of the following is true about cell wall of gram-positive bacteria?	It consists of multiple layers	It is thicker than that associated with gram-negative bacteria	It contains teichoic acids	Less lipid	It contains teichoic acids
32	The cell walls of many gram positive bacteria can be easily destroyed by the enzyme known	lipase	Lysozyme	pectinase	peroxidase	lysozyme

	as					
33	For most bacteria, the optimum pH for growth lies between	6.5-7.5	3.5-4.5	4.5-5.5	5.5-6.5	6.5-7.5
34	Suspension cultures consist of cells and cell aggregates, growing dispersed in	liquid medium	solid nutrient medium	solid or liquid medium	none of these	liquid medium
35	The protoplast can be used to	modify genetic information	create plant hybrid	study plant viral infections	no alteration	modify genetic information
36	The cell wall of	gram-positive bacteria are thicker than gram-negative bacteria	gram-negative bacteria are thicker than gram-positive bacteria	both have same thickness but composition is different	less lipid	gram-positive bacteria are thicker than gram-negative bacteria
37	Peptidoglycan is also known as	N-acetyl muramic acid	murein mucopeptide	N acetylglucosamine	mesodiaminopimetic acid	murein mucopeptide
38	The cocci which forms a pair	Staphylococci	Diplococci	Tetracocci	Streptococci	diplococci
39	Chemotaxis is a phenomenon of	swimming away of bacteria	swimming towards bacteria	swimming away or towards of bacteria in presence of chemical compound	swim in media	swimming away or towards of bacteria in presence of chemical compound
40	The structure responsible for motility of bacteria is	pilli	Flagella	sheath	capsules	flagella

41	The next to last step in peptidoglycan biosynthesis is	synthesis of the NAM-peptide subunit	removal of the subunit from bactoprenol	linking the sugar of the disaccharide-peptide unit to the growing peptidoglycan chain	cross-linking the peptide side chains of peptidoglycan	linking the sugar of the disaccharide-peptide unit to the growing peptidoglycan chain
42	The cocci which forms a chain is	Streptococci	Diplococci	Staphylococci	Tetracocci	Streptococci
43	The arrangement, in which flagella are distributed all round the bacterial cell, is known as	lophotrichous	Amphitrichous	peritrichous	monotrichous	peritrichous
44	Periplasm is	the area between the inner and outer membranes of gram-negative bacteria	the area between the inner and outer membranes of Gram-positive bacteria	the interior portion of mitochondria	the area outside the cell membrane that is influenced by the polymers	the area between the inner and outer membranes of gram-negative bacteria
45	Which of the following has peptidoglycan as a major constituent of cell wall?	Gram-negative bacteria	Gram-positive bacteria	Fungi	virus	Gram-positive bacteria
46	The common word for bacteria which are helically curved rods is	cooci	Pleomorphic	bacillus	spirilla	spirilla
47	The bacteria deficient in cell wall is	Treponema	Mycoplasma	Staphylococcus	Klebsiella	Mycoplasma
48	Which of the following is not true about peptidoglycan?	It is a polymer consisting of N-acetyl	It is present in prokaryotic cell	It occurs in the form of a bag	No NAM NAG	No NAM NAG

		glucosamine, N-acetyl muramic acid and amino acids (alanine, lysine, etc.)	wall	shaped macro molecule surrounding the cytoplasm membrane		
49	Single or clusters of flagella at both poles is known as	monotrichous	Peritrichous	amphitrichous	atrichous	amphitrichous
50	Which of the following bacterial genera (that produces endospore) have medical importance?	Salmonella	Bacillus	proteus	E. coli	Bacillus
51	_____media is used for cultivation of bacteria	Nutrient agar	MacConkey agar	EMB agar	MHA	Nutrient agar
52	Single bacteria will form a _____ colony	Multiple	Single	No	infinite	Single
53	Which instrument is used for sterilization above 100° C	Flame	Autoclave	Filters	Desiccators	Autoclave
54	_____ is the first phase in growth curve	Log	Lag	stationary	death	Lag
55	DNA to DNA is called as	replication	Biosynthesis	translation	transcription	replication
56	Oligonucleotide means containing	10 nucleotides	more than 10 nucleotides	less than 10 nucleotides	no nucleotides	10 nucleotides
57	_____ group of bacteria grows in high temperature	Halophiles	Basophiles	thermophiles	psychrophiles	thermophiles
58	The group of gram positive bacteria having low G+C contents	cyanobacteria	Nanobacteria	Firmicutes	Actinobacteria	Firmicutes

	are called as					
59	BGA expanded as	Blue Green Algae	Blue Grown Algae	Blue non Grown Algae	Blue Gram Algae	Blue Green Algae
60	The time required to kill 90% of the microorganisms in a sample at a specific temperature is the	decimal reduction time	thermal death point	F value	D value	decimal reduction time

Unit IV

Syllabus

Brief account of photosynthetic and accessory pigments. Phytoplanktons and Zooplanktons, Red tides, Zones, Bioluminescence and Biopigment, Marine micro and macro organisms, Coral reefs, Mangrooves, Hydrothermal vents and water currents.

Photosynthetic Pigments

Pigments are colorful compounds.

Pigments are chemical compounds which reflect only certain wavelengths of visible light. This makes them appear "colorful". Flowers, corals, and even animal skin contain pigments which give them their colors. More important than their reflection of light is the ability of pigments to **absorb** certain wavelengths.

Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other **autotrophs** --organisms which make their own food using **photosynthesis**. In plants, algae, andcyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture more of the sun's energy.

There are three basic classes of pigments.

Chlorophylls are greenish pigments which contain a **porphyrin ring**. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.

There are several kinds of chlorophyll, the most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in the plants. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. The differences between the chlorophylls of these major groups was one of the first clues that they were not as closely related as previously thought.

Carotenoids are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their color. These compounds are composed of two small six-carbon

rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called **accessory pigments**. One very visible accessory pigment is **fucoxanthin** the brown pigment which colors kelps and other brown algae as well as the diatoms.

Phycobilins are water-soluble pigments, and are therefore found in the cytoplasm, or in the stroma of the chloroplast. They occur only inCyanobacteria and Rhodophyta. two classes of phycobilins which may be extracted from these "algae". The vial on the left contains the bluish pigment**phycocyanin**, which gives the Cyanobacteria their name. The vial on the right contains the reddish pigment **phycoerythrin**, which gives the red algae their common name.

Phycobilins are not only useful to the organisms which use them for soaking up light energy; they have also found use as research tools. Both pycocyanin and phycoerythrin **fluoresce** at a particular wavelength. That is, when they are exposed to strong light, they absorb the light energy, and release it by emitting light of a very narrow range of wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are chemically bonded to antibodies, which are then put into a solution of cells. When the solution is sprayed as a stream of fine droplets past a laser and computer sensor, a machine can identify whether the cells in the droplets have been "tagged" by the antibodies. This has found extensive use in cancer research, for "tagging" tumor cells.

Plants

Green plants have six closely related photosynthetic pigments (in order of increasing polarity):

Carotene - an orange pigment

Xanthophyll - a yellow pigment

Phaeophytin *a*https://en.wikipedia.org/wiki/Photosynthetic_pigment - cite_note-phaeophytin-1 - a gray-brown pigment

Phaeophytin *b*- a yellow-brown pigment

Chlorophyll *a* - a blue-green pigment

Chlorophyll *b* - a yellow-green pigment

Chlorophyll a is the most common of the six, present in every plant that performs photosynthesis. The reason that there are so many pigments is that each absorbs light more efficiently in a different part of the electromagnetic spectrum. Chlorophyll a absorbs well at a wavelength of about 400-450 nm and at 650-700 nm; chlorophyll b at 450-500 nm and at 600-650 nm. Xanthophyll absorbs well at 400-530 nm. However, none of the pigments absorbs well in the green-yellow region, which is responsible for the abundant green we see in nature.

Bacteria

Like plants, the cyanobacteria use water as an electron donor for photosynthesis and therefore liberate oxygen; they also use chlorophyll as a pigment. In addition, most cyanobacteria use phycobiliproteins, water-soluble pigments which occur in the cytoplasm of the chloroplast, to capture light energy and pass it on to the chlorophylls. (Some cyanobacteria, the prochlorophytes, use chlorophyll b instead of phycobilin.) It is thought that the chloroplasts in plants and algae all evolved from cyanobacteria.

Several other groups of bacteria use the bacteriochlorophyll pigments (similar to the chlorophylls) for photosynthesis. Unlike the cyanobacteria, these bacteria do not produce oxygen; they typically use hydrogen sulfide rather than water as the electron donor.

Recently, a very different pigment has been found in some marine γ -proteobacteria: proteorhodopsin. It is similar to and probably originated from bacteriorhodopsin (see below under archaea). Bacterial chlorophyll b has been isolated from Rhodopseudomonas spp. but its structure is not yet known

Algae

Green algae, red algae and glaucophytes all use chlorophylls. Red algae and glaucophytes also use phycobiliproteins, but green algae do not.

Archaea

Halobacteria use the pigment bacteriorhodopsin which acts directly as a proton pump when exposed to light.

Photosynthesis

The primary source of energy for nearly all life is the Sun. The energy in sunlight is introduced into the biosphere by a process known as photosynthesis, which occurs in plants, algae and some types of bacteria. Photosynthesis can be defined as the physico-chemical process by which photosynthetic organisms use light energy to drive the synthesis of organic compounds.

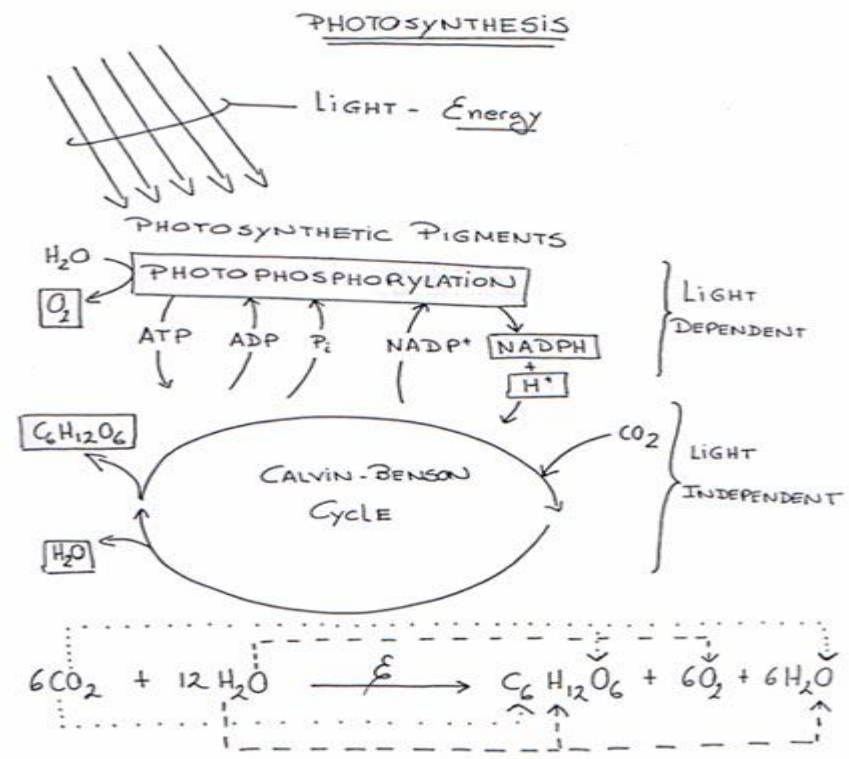
Virtually all oxygen in the atmosphere is thought to have been generated through the process of photosynthesis It is a very complicated biological system. Basically it is the process that converts energy from sunlight to chemical forms of energy that can be used.

Plants, algae, as well as cyanobacteria are responsible for a major part of photosynthesis in oceans. These organisms convert CO2 (carbon dioxide) to organic material by reducing this gas to carbohydrates in a rather complex set of reactions. Electrons for this reduction reaction ultimately come from water, which is then converted to oxygen and protons. Energy for this process is provided by light, which is absorbed by pigments (primarily chlorophylls and carotenoids). Chlorophylls absorb blue and red light and carotenoids absorb blue-green light , but green and yellow light are not effectively absorbed by photosynthetic pigments in plants; therefore, light of these colors is either reflected by leaves or passes through the leaves.

why plants are green. Other photosynthetic organisms, such as cyanobacteria, known as blue-green algae, and red algae, have additional pigments called phycobilins that are red or blue and that absorb the colors of visible light that are not effectively absorbed by chlorophyll and carotenoids. Yet other organisms, such as the purple and green bacteria, contain bacteriochlorophyll that absorbs in the infrared, in addition to in the blue part of the spectrum. These bacteria do not evolve oxygen, but perform photosynthesis under anaerobic (oxygen-less) conditions. All plants, algae, and cyanobacteria which photosynthesize contain the pigment chlorophyll "a." A second kind of chlorophyll is chlorophyll "b", which occurs only in green algae" and in the plants. A third form of chlorophyll which is common is called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates.

Photosynthetic pigments come in a huge variety. Some are chlorophyll, carotenoids, and phycobilins, and they differ from each other in their precise chemical structure. Light energy is absorbed by individual pigments, but is not used immediately by these pigments for energy conversion. Instead, the light energy is transferred to chlorophylls that are in a special protein environment where the actual energy conversion event occurs, the light energy is used to transfer an electron to a neighboring pigment. The purpose is to maintain a high rate of electron transfer in the reaction center, even at lower light intensities.

Plants have developed means to convert some of the absorbed light energy to heat rather than to use the absorbed light necessarily for photosynthesis. Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other autotrophs--organisms which make their own food using photosynthesis. White light is separated into the different colors (wavelengths) of light by passing it through a prism. Wavelength is defined as the distance from peak to peak. Energy is inversely proportional to the wavelength: longer wavelengths have less energy than do shorter ones. Without photosynthesis, the oxygen in the atmosphere would be depleted within several thousand years. It should be emphasized that plants respire just like any other higher organism, and that during the day this respiration is masked by a higher rate of photosynthesis.



In eukaryotes, photosynthesis takes place in the chloroplast, which has long been known to have prokaryotic features. Chloroplasts are thought to have evolved from a cyanobacterium (or close relative) that was in a symbiotic relationship with a eukaryotic, non-photosynthetic cell and was engulfed inside this cell. The cyanobacterium and the eukaryotic cell presumably were in a mutually beneficial relationship (endosymbiosis), with the photosynthetic organism sharing some of its produced carbohydrates with the host, and the host providing the photosynthetic bacterium with other compounds. The prokaryote slowly gave up its independence as well as its cell wall, and some of its genetic information was transferred to the nucleus of its eukaryotic host. The resulting chloroplast maintains a small, prokaryote-like circular DNA of its own (DNA is material carrying genetic information); this DNA contains the genetic blueprint to make many of the membrane proteins needed in the chloroplast, which apparently are not easily targeted to and/or transported into the chloroplast. Occasionally, photosynthetic organisms are found where the chloroplast has retained a little more of the original cyanobacterial features. For example, in algae such as Cyanophora paradoxa plastids (called cyanelles)

are found that resemble cyanobacteria in their overall morphology as well as in the fact that they are surrounded by a cell wall. Even though plants are the most visible representatives of photosynthetic organisms, it should be emphasized that many other types of photosynthetic organisms exist.

Accessory pigments are light-absorbing compounds, found in photosynthetic organisms, that work in conjunction with chlorophyll *a*. They include other forms of this pigment, such as chlorophyll *b* in green algal and higher plant antennae, while other algae may contain chlorophyll *c* or *d*. In addition, there are many non-chlorophyll accessory pigments, such as carotenoids or phycobiliproteins, which also absorb light and transfer that light energy to photosystem chlorophyll. Some of these accessory pigments, in particular the carotenoids, also serve to absorb and dissipate excess light energy, or work as antioxidants. The large, physically associated group of chlorophylls and other accessory pigments is sometimes referred to as a *pigment bed*, though this term is no longer supported by what we know of photosystem and antenna complex structures.

The different chlorophyll and non-chlorophyll pigments associated with the photosystems all have different absorption spectra, either because the spectra of the different chlorophyll pigments are modified by their local protein environment or because the accessory pigments have intrinsic structural differences. The result is that, in vivo, a composite absorption spectrum of all these pigments is broadened and flattened such that a wider range of visible and infrared radiation is absorbed by plants and algae. Most photosynthetic organisms do not absorb green light well, thus most remaining light under leaf canopies in forests or under water with abundant plankton is green, a spectral effect called the "green window". Organisms such as some cyanobacteria and red algae contain accessory phycobiliproteins that absorb green light reaching these habitats.

In aquatic ecosystems, it is likely that the absorption spectrum of water, along with gilvin and tripton (dissolved and particulate organic matter, respectively), determinesphototrophic niche differentiation. The six shoulders in the light absorption of water between wavelengths 400 and 1100 nm correspond to troughs in the collective absorption of at least twenty diverse species of phototrophic bacteria. Another effect is due to the overall trend for water to absorb low frequencies, while gilvin and tripton absorb higher ones. This is

why open ocean appears blue and supports yellow species such as *Prochlorococcus*, which contains divinyl- chlorophyll *a* and *b*. *Synechococcus*, colored red withphycoerythrin, is adapted to coastal bodies, while phycocyanin allows Cyanobacteria to thrive in darker inland waters.

Anabolism (from Greek: ἀνά, "upward" and βάλλειν, "to throw") is the set of metabolic pathways that construct molecules from smaller units.[1] These reactions require energy. One way of categorizing metabolic processes, whether at the cellular, organ or organism level, is as "anabolic", or as "catabolic" which is the opposite. Anabolism is powered by catabolism, where large molecules are broken down into smaller parts and then used up in cellular respiration. Many anabolic processes are powered by the hydrolysis of adenosine triphosphate (ATP).

Anabolic processes tend toward "building up" organs and tissues. These processes produce growth and differentiation of cells and increase in body size, a process that involvessynthesis of complex molecules. Examples of anabolic processes include the growth and mineralization of bone and increases in muscle mass. Endocrinologists have traditionally classified hormones as anabolic or catabolic, depending on which part of metabolism they stimulate. The classic anabolic hormones are the anabolic steroids, which stimulate protein synthesis, muscle growth, and insulin. The balance between anabolism and catabolism is also regulated by circadian rhythms, with processes such as glucose metabolism fluctuating to match an animal's normal periods of activity throughout the day

Autotrophy (in certain plants and bacteria) the process of making food from inorganic substances, using photosynthesis

au·to·troph

An organism capable of synthesizing its own food from inorganic substances, using light or chemical energy . Green plants, algae, and certain bacteria are autotrophs.

An **autotroph**[α] ("self-feeding", from the Greek *autos* "self" and *trophe* "nourishing") or **producer**, is an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in its surroundings, generally using energy from light (photosynthesis) or inorganic chemical reactions (chemosynthesis). They are the producers in a food chain, such as plants on land or algae in water, in contrast to heterotrophs as consumers of autotrophs. They do not need a living source of energy or organic carbon. Autotrophs can reduce carbon dioxide to

make organic compounds for biosynthesis and also create a store of chemical energy. Most autotrophs use water as the reducing agent, but some can use other hydrogen compounds such as hydrogen sulfide. Some autotrophs, like green plants and algae, are phototrophs, which means they convert electromagnetic energy from sunlight into chemical energy in the form ofreduced carbon.

Autotrophs can be photoautotrophs or chemoautotrophs. Phototrophs use light as an energy source, while chemotrophs utilize electron donors as a source of energy, whether from organic or inorganic sources; however in the case of autotrophs, these electron donors come from inorganic chemical sources. Such chemotrophs are lithotrophs. Lithotrophs use inorganic compounds, such as hydrogen sulfide, elemental sulfur, ammonium and ferrous iron, as reducing agents for biosynthesis and chemical energy storage. Photoautotrophs and lithoautotrophs use a portion of the ATP produced during photosynthesis or the oxidation of inorganic compounds to reduce $NADP^+$ to NADPH to form organic compounds.

The German term *autotroph* was coined by Albert Bernhard Frank in 1892

Variants

Some organisms rely on organic compounds as a source of carbon, but are able to use light or inorganic compounds as a source of energy. Such organisms are not defined as autotrophic, but rather as heterotrophic. An organism that obtains carbon from organic compounds but obtains energy from light is called a ***photoheterotroph***, while an organism that obtains carbon from organic compounds but obtains energy from the oxidation of inorganic compounds is termed ***chemoheterotroph***, ***chemolithoheterotroph***, or ***lithoheterotroph***.

Evidence suggests that some fungi may also obtain energy from radiation. Such radiotrophic fungi were found growing inside a reactor of the Chernobyl nuclear power plant.

Ecology

Autotrophs are fundamental to the food chains of all ecosystems in the world. They take energy from the environment in the form of sunlight or inorganic chemicals and use it to create energy-rich molecules such as carbohydrates. This mechanism is called primary production. Other organisms, called

heterotrophs, take in autotrophs as food to carry out functions necessary for their life. Thus, heterotrophs — all animals, almost all fungi, as well as most bacteria and protozoa — depend on autotrophs, or primary producers, for the energy and raw materials they need. Heterotrophs obtain energy by breaking down organic molecules (carbohydrates, fats, and proteins) obtained in food. Carnivorous organisms rely on autotrophs indirectly, as the nutrients obtained from their heterotroph prey come from autotrophs they have consumed.

Most ecosystems are supported by the autotrophic primary production of plants that capture photons initially released by the sun. The process of photosynthesis splits a water molecule (H_2O), releasing oxygen (O_2) into the atmosphere, and reducing carbon dioxide (CO_2) to release the hydrogen atoms that fuel the metabolic process of primary production. Plants convert and store the energy of the photon into the chemical bonds of simple sugars during photosynthesis. These plant sugars are polymerized for storage as long-chain carbohydrates, including other sugars, starch, and cellulose; glucose is also used to make fats and proteins. When autotrophs are eaten by heterotrophs, i.e., consumers such as animals, the carbohydrates, fats, and proteins contained in them become energy sources for the heterotrophs.Proteins can be made using nitrates, sulfates, and phosphates in the soil.

Bacterial Photosynthesis

Many prokaryotes (bacteria and cyanobacteria) possess phototrophic modes of metabolism. The types of photosynthesis in the two groups of prokaryotes differ mainly in the type of compound that serves as the hydrogen donor in the reduction of CO_2 to glucose. Phototrophic organisms differ from heterotrophic organisms in that they utilize the glucose synthesized intracellularly for biosynthetic purposes (as in starch synthesis) or for energy production, which usually occurs through cellular respiration.

Unlike phototrophs, heterotrophs require glucose (or some other preformed organic compound) that is directly supplied as a substrate from an exogenous source. Heterotrophs cannot synthesize large concentrations of glucose from $CO_2$by specifically using H_2O or (H_2S) as a hydrogen source and sunlight as energy. Plant metabolism is a classic example of photolithotrophic metabolism: plants need CO_2 and sunlight; H_2O must be provided as a hydrogen source and usually NO_3^- is the nitrogen source for protein synthesis. Organic nitrogen, supplied as fertilizer, is converted to NO_3^- in all soils by bacteria via the process of ammonification and nitrification. Although plant cells are phototrophic, they also exhibit a heterotrophic mode of metabolism in that they respire. For example, plants use classic respiration to catabolize glucose that

is generated photosynthetically. Mitochondria as well as the soluble enzymes of the glycolytic pathway are required for glucose dissimilation, and these enzymes are also found in all plant cells. The soluble Calvin cycle enzymes, which are required for glucose synthesis during photosynthesis, are also found in plant cells. It is not possible to feed a plant by pouring a glucose solution on it, but water supplied to a plant will be "photolysed" by chloroplasts in the presence of light; the hydrogen(s) generated from H_2O is used by Photosystems I and II (PSI and PSII) to reduce $NADP^+$ to NADPH + H^+. With the ATP generated by PSI and PSII, these reduced pyridine nucleotides, CO_2 is reduced intracellularly to glucose. This metabolic process is carried out in an integrated manner by Photosystems I and II ("Z" scheme) and by the Calvin cycle pathway. A new photosynthetic, and nitrogen fixing bacterium, *Heliobacterium chlorum*, staining Gram positive was isolated, characterized, and found to contain a new type of chlorophyll, i.e., bacteriochlorophyll 'g'. 16S r-RNA sequence analyses showed this organism to be phylogenetically related to members of the family *Bacillaceae*, although all currently known phototrophes are Gram negative(Table 4.4). A few*Heliobacteriium* strains did show the presence of endospores. Another unusual phototrophe is the Gram negative*Halobacterium halobium* (now named *Halobacterium salinarium*), an archaebacterium growing best at 30°C in 4.0–5.0 M (or 25%, w/v) NaCl. This bacterium is a facultative phototrophe having a respiratory mode; it also possesses a purple membrane within which bacteriorhodopsin serves as the active photosynthetic pigment. This purple membranae possesses a light driven proton translocation pump which mediates photosynthetic ATP synthesis via a proton extrusion reaction (see Mitchell Hypothesis). Table 4-4 summarizes the characteristics of known photosynthetic bacteria.

Characteristics Commonly Exhibited by Phototrophic Bacteria.

Autotrophy

Bacteria that grow solely at the expense of inorganic compounds (mineral ions), without using sunlight as an energy source, are called autotrophs, chemotrophs, chemoautotrophs, or chemolithotrophs. Like photosynthetic organisms, all autotrophs use CO_2 as a carbon source for growth; their nitrogen comes from inorganic compounds such as NH_3, NO_3^-, or N_2 Interestingly, the energy source for such organisms is the oxidation of specific inorganic compounds. Which inorganic compound is oxidized depends on the bacteria in question (Table 4-5). Many autotrophs will not grow on media that contain organic matter, even agar.

TABLE 4-5 Inorganic Oxidation Reactions Used by Autotrophic Bacteria as Energy Sources

Chemosynthetic Type	Inorganic Compounds Oxidized as Energy (~E) Source	Representative Families, Genera, and Species[a]	Nitrogen Cycle Reaction
NH_3 oxidizers (aerobic)	$NH_3 \rightarrow NO_2$ (~E) (~E) = chemical energy or ATP produced	Nitrobacteriaceae (*Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*)	Nitrification Nitrification Nitrification Nitrification
NO_2^- oxidizers (aerobic)	$NO_2^- \rightarrow NO_3^-$ (~E)	Nitrobacteriaceae (*Nitrobacter*, *Nitrococcus*)	Nitrification Nitrification Nitrification
Sulfur oxidizers[b] (aerobic) Iron oxidizers (aerobic)	$S^- \rightarrow SO_4^{2-}$ (~E) $Fe^{2+} \rightarrow Fe^{3+}$ (~E) use both reactions	*Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, *Ferrobacillus*, *Leptothrix*	
Sulfur-compound oxidizers Denitrification (anaerobic)	S_2O_3 oxidized; NO_3^- reduced	*Thiobacillus denitrificans*	

[a] All are Gram-negative species (see *Bergey's Manual of Determinative Bacteriology*, 8th ed, part 12).
[b] Strict autotrophic modes of metabolism are not present in sulfur and sulfur compound-oxidizing bacteria. For example, heterotrophic sulfur compound oxidizers are known, the aerobic species being able to oxidize $H_2S \rightarrow S_2$ (~E) (e.g., *Beggiatoa* and *Thiothrix* species).

Table 4-5 Inorganic Oxidation Reactions Used by Autotrophic Bacteria as Energy Sources.

Also found among the autotrophic microorganisms are the sulfur-oxidizing or sulfur-compound-oxidizing bacteria, which seldom exhibit a strictly autotrophic mode of metabolism like the obligate nitrifying bacteria (see discussion of nitrogen cycle below). The representative sulfur compounds oxidized by such bacteria are H_2S, S_2, and S_2O_3. Among the sulfur bacteria are two very interesting organisms; *Thiobacillus ferrooxidans*, which gets its energy for autotrophic growth by oxidizing elemental sulfur or ferrous iron, and *T. denitrificans*, which gets its energy by oxidizing S_2O_3anaerobically, using NO_3^- as the sole terminal electron acceptor. T denitrificans reduces NO_3 to molecular N_2, which is liberated as a gas; this biologic process is called denitrification.

All autotrophic bacteria must assimilate CO_2, which is reduced to glucose from which organic cellular matter is synthesized. The energy for this biosynthetic process is derived from the oxidation of inorganic compounds discussed in the previous paragraph. Note that all autotrophic and phototrophic bacteria possess essentially the same organic cellular constituents found in heterotrophic bacteria; from a nutritional viewpoint, however, the autotrophic mode of metabolism is unique, occurring only in bacteria.

Anerobic Respiration

Some bacteria exhibit a unique mode of respiration called anaerobic respiration. These heterotrophic bacteria that will not grow anaerobically unless a specific chemical component, which serves as a terminal electron acceptor, is added to the medium. Among these electron acceptors are NO_3^-, SO_4^{2-}, the organic compound fumarate, and CO_2. Bacteria requiring one of these compounds for anaerobic growth are said to be anaerobic respirers.

A large group of anaerobic respirers are the nitrate reducers (Table 4-6). The nitrate reducers are predominantly heterotrophic bacteria that possess a complex electron transport system(s) allowing the NO_3^- ion to serve anaerobically as a terminal acceptor of electrons

Red tides and Zones

Red tide is a common name for a worldwide phenomenon known as an algae bloom (large concentrations of aquatic microorganisms—protozoans or unicellular algae) when it is caused by species of dinoflagellates and other algae. The upwelling of nutrients from the sea floor from massive storms is most likely the cause of these events.

Certain species of phytoplankton and dinoflagellates found in red tides contain photosynthetic pigments that vary in color from brown to red. When the algae are present in high concentrations, the water appears to be discolored or murky, varying in color from a rust color to pink to blood red. Specifically, red tide species can be found in oceans, bays, and places where fresh water meets salt water, but they can not thrive in freshwater environments due to the lack of salinity. The growth of the algal bloom depends on wind, temperature, nutrients, and salinity. Some red tide algal blooms are associated with fish kills. The production of natural toxins such as brevetoxins and ichthyotoxins are harmful to marine life. Generally, red tides are described as harmful algal blooms. The most conspicuous effects of these kinds of red tides are the associated wildlife mortalities, as well as harmful human exposure.

List of common red tide genera

Gonyaulax

Karenia

Gymnodinium

Dinophysis

Noctiluca

Chattonella

Amoebophyra

Harmful toxins produced by the red tide

Marine life exposure

Red tides occur naturally off coasts all over the world. Not all red tides have toxins or are harmful. Where red tides occur, dead fish wash up on shore for up to two weeks after a red tide has been through the area. In addition to killing fish, the toxic algae contaminate shellfish. Some mollusks are not susceptible to the toxin, and store it in their fatty tissues. Shellfish consume the organisms responsible for red tide and concentrate saxitoxin(produced from these organisms) in their tissues. Saxitoxin blocks sodium channels and ingestion can cause paralysis within 30 minutes.[5] Other animals that eat the shellfish are susceptible to the neurotoxin, leading to neurotoxic shellfish poisoning and sometimes even death. Most mollusks and clams filter feed, which results in higher concentrations of the toxin than just drinking the water. Scaup, for example, are diving ducks whose diet mainly consists of mollusks. When scaup eat the filter-feeding shellfish that are concentrated with high levels of the red tide toxin, their population along with other types of diving ducks become a prime target for poisoning. However, even birds that do not eat

mollusks can be affected by simply eating dead fish on the beach or drinking the water, as in the Peking duck experiment. The toxins released by the blooms can kill marine animals

including dolphins, sea turtles, birds, and manatees. Marine dinoflagellates produce ichthyotoxins. Fish such as Atlantic herring, American pollock, winter flounder, Atlantic salmon, and cod were dosed orally with these toxins in an experiment. Within minutes of receiving doses of the toxin, fish started to exhibit a loss of equilibrium and began to swim in an irregular, jerking pattern followed by paralysis and shallow,

arrhythmic breathing and eventually death after about an hour. Scientists concluded that the toxic red tide had negative effects on fish that were exposed to it.

Human exposure

Humans are affected by the red tide species by ingesting improperly harvested shellfish, breathing in aerosolized brevetoxins (i.e. PbTx or *Ptychodiscus* toxins) and in some cases skin contact. The brevetoxins bind to voltage-gated sodium channels, important structures of cell membranes. Binding results in persistent activation of nerve cells, which interferes with neural transmission leading to health problems. These toxins are created within the unicellular organism, or as a metabolic product. The two major types of brevetoxin compounds have similar but distinct backbone structures. PbTx-2 is the primary intracellular brevetoxin produced by *K. brevis* blooms. However, over time, the PbTx-2 brevetoxin can be converted to PbTx-3 through metabolic changes. Researchers found that PbTx-2 has been the primary intracellular brevetoxin that converts overtime into PbTx-3. When the cells rupture, they release extracellular brevetoxins into the environment. Some of those stay in the ocean, while other particles get aerosolized. During onshore winds, brevetoxins can become aerosolized by bubble-mediated transport, causing respiratory irritation, bronchoconstriction, coughing, and wheezing among other symptoms. On a windy day, avoiding contact with the aerosolized toxin is recommended. These individuals report a decrease in respiratory function after only 1 hour of exposure to a *K. brevis* red-tide beach and these symptoms may last for days. People with severe or persistent respiratory conditions (such as chronic lung disease or asthma) may experience stronger adverse reactions. The National Oceanic and Atmospheric Administration's National Ocean Service provides a public conditions report identifying possible respiratory irritation impacts in areas affected by red tides.

In most cases like in the U.S., the seafood consumed by humans is tested regularly for toxins by the USDA to ensure safe consumption. However, improper harvesting of shellfish can cause paralytic shellfish poisoning and neurotoxic shellfish Poisoning in humans. Some symptoms include drowsiness, diarrhea, nausea, loss of motor control, tingling, numbing or aching of extremities, incoherence, and respiratory paralysis. Lastly, reports of skin irritation after swimming in the ocean during a red tide are common, so people should try to avoid the red tide when it is in the area.

Red tide

The dinoflagellate labeled above is the microscopic alga *Karenia brevis*. It is the cause of red tide in the Gulf of Mexico. The algae propel themselves using a longitudinal flagellum (A) and a transverse flagellum

(B). The longitudinal flagellum lies in a groove-like structure called the cingulum (F). The dinoflagellate is separated into an upper portion called the epitheca (C) where the apical horn resides (E) and a lower portion called the hypotheca (D).

Red tide is a colloquial term used to refer to one of a variety of natural phenomena known as harmful algal blooms. The term specifically refers to blooms of a species of dinoflagellate.[18] It is being phased out among researchers because:

Red tides are not necessarily red and many have no discoloration at all.

They are unrelated to movements of the tides.

The term is imprecisely used to refer to a wide variety of algal species that are known as bloom-formers.

As a technical term, it is being replaced in favour of more precise terminology, including the generic term "harmful algal bloom" for harmful species, and "algal bloom" for benign species.

On the U.S. coasts

The term red tide is most often used in the US to refer to *Karenia brevis* blooms in the eastern Gulf of Mexico, also called the Florida red tide. *K. brevis* is one of many different species of the genus *Karenia* found in the world's oceans. Major advances have occurred in the study of dinoflagellates and their genomics. Some include identification of the toxin-producing genes (*PKS* genes), exploration of environmental changes (temperature, light/dark, etc.) have on gene expression, as well as an appreciation of the complexity of the *Karenia* genome. These blooms have been documented since the 1800s, and occur almost annually along Florida's coasts. There was increased research activity of HABs in the 1980s and

1990s. This was primarily driven by media attention from the discovery of new HAB organisms and the potential adverse health effects of their exposure to animals and humans. The Florida red tides have been observed to have spread as far as the eastern coast of Mexico. The density of these organisms during a bloom can exceed tens of millions of cells per litre of seawater, and often discolor the water a deep reddish-brown hue.

Red tide is also sometimes used to describe harmful algal blooms on the northeast coast of the United States, particularly in the Gulf of Maine. This type of bloom is caused by another species of dinoflagellate known as *Alexandrium fundyense*. These blooms of organisms cause severe disruptions in fisheries of these waters, as the toxins in these organism cause filter-feeding shellfish in affected waters to become poisonous for human consumption due to saxitoxin. The related *Alexandrium monilatum* is found in subtropical or tropical shallow seas and estuaries in the western Atlantic Ocean, the Caribbean Sea, the Gulf of Mexico, and the eastern Pacific Ocean.

Factors that may contribute to a bloom

Red tide (NOAA)

Red tides contain dense concentrations of organisms and appear as discolored water, often reddish-brown in color. It is a natural phenomenon, but the exact cause or combination of factors that result in a red tide outbreak are not necessarily known. However, three key factors are thought to play an important role in a bloom - salinity, temperature, and wind. Red tides cause economic harm, so outbreaks are carefully monitored. For example, the Florida Fish and Wildlife Conservation Commission provides an up-to-date status report on red tides in Florida. The Texas Parks and Wildlife Department also provides a status report. While no particular cause of red tides has been found, many different factors can contribute to their presence. These factors can include water pollution, which originates from sources such as human sewage and agricultural runoff. There are other factors that have been associated with the increase in red tides, such as weather, climate change, and tidal patterns, although the correlation is not always quite clear. Red tide algal blooms tend to be more frequent during the summer because of the warm temperatures.

The occurrence of red tides in some locations appears to be entirely natural (algal blooms are a seasonal occurrence resulting from coastal upwelling, a natural result of the movement of certain ocean currents) while in others they appear to be a result of increased nutrient pollution from human activities. The growth of marine phytoplankton is generally limited by the availability of nitrates and phosphates, which can be abundant in agricultural run-off as well as coastal upwelling zones.

Coastal water pollution produced by humans and systematic increase in seawater temperaturehave also been implicated as contributing factors in red tides. Other factors such as iron-rich dust influx from large desert areas such as the Sahara Desert are thought to play a major role in causing red tides. Some algal blooms on the Pacific Coast have also been linked to occurrences of large-scale climatic oscillations such as El Niño events. While red tides in the Gulf of Mexico have been occurring since the time of early explorers such as Cabeza de Vaca, what initiates these blooms and how large a role anthropogenic and natural factors play in their development is unclear. Whether the apparent increase in frequency and severity of algal blooms in various parts of the world is in fact a real increase or is due to increased observation effort and advances in species identification methods is also debated.

While the human contribution to the long-term increase in red tides is apparent, some researchers propose that climate change is also a factor, with more research still needed to claim it as a definitive cause. Increasing temperature, enhanced surface stratification, alteration of ocean currents, intensification or weakening of local nutrient upwelling, stimulation of photosynthesis by elevated CO_2, reduced calcification through ocean acidification, and heavy precipitation and storm events causing changes in land runoff and micronutrient availability may all produce contradictory species- or even strain-specific responses. In terms of harmful algal blooms (HABs), we can expect: (i) range expansion of warm-water species at the expense of cold-water species, which are driven poleward; (ii) species-specific changes in the abundance and seasonal window of growth of HAB taxa; (iii) earlier timing of peak production of some phytoplankton; and (iv) secondary effects for marine food webs, notably when individual zooplankton and fish grazers are differentially impacted by climate change. However, the potential consequences of these changes for HABs have received relatively little attention and are not well

understood. Substantial research is needed to evaluate the direct and indirect associations between HABs, climate change, ocean acidification, and human health.

A multi-partner project funded by the federal *EcoHab* program (NOAA) and published by the Mote Marine Laboratory shows a list of what feeds red tides. A study from the Florida FWC shows the *Karenia brevis* algae red tide found in Florida is fed and worsened by nitrogen (N) and phosphorus (P).

Z o n e s :

History

Shrimp trawlers first reported a 'dead zone' in the Gulf of Mexico in 1950, but it was not until 1970 when the size of the hypoxic zone had increased that scientists began to investigate.

After 1950, the conversion of forests and wetlands for agricultural and urban developments accelerated. "Missouri River Basin has had hundreds of thousands of acres of forests and wetlands (66,000,000 acres) replaced with agriculture activity [. . .] In the Lower Mississippi one-third of the valley's forests were converted to agriculture between 1950 and 1976."

In July 2007, a dead zone was discovered off the coast of Texas where the Brazos River empties into the Gulf.[37]

Gulf of Oman

In 2018, scientists confirmed the Gulf of Oman encompasses one of the world's largest and most severe marine dead zones. The dead zone encompasses nearly the entire 63,700-square-mile Gulf of Oman. The dead zone consists entirely of anoxic conditions, meaning no oxygen is present, or suboxic conditions, with low oxygen levels. The cause is a combination of increased ocean warming with increased runoff of nitrogen and phosphorus from fertilizers. The dead zone had previously been unstudied due to geopolitical factors.[38]

Economic impact

Some assert that the dead zone threatens lucrative commercial and recreational fisheries in the Gulf of Mexico. "In 2009, the dockside value of commercial fisheries in the Gulf was $629 million. Nearly three million recreational fishers further contributed about $10 billion to the Gulf economy, taking 22 million fishing trips." Scientists are not in universal agreement that nutrient loading has a negative impact on fisheries. Grimes makes a case that nutrient loading enhances the fisheries in the Gulf of Mexico. Courtney et al. hypothesize, that nutrient loading may have contributed to the increases in red snapper in the northern and western Gulf of Mexico.

Size

The area of hypoxic bottom water that occurs for several weeks each summer in the Gulf of Mexico has been mapped most years from 1985 through 2017. The size varies annually from a record high in 2017 when it encompassed more than 22,730 sq kilometers (8,776 square miles) to a record low in 1988 of 39 sq kilometers (15 square miles). The 2015 dead zone measured 16,760 square kilometers (6,474 square miles).

In late summer 1988 the dead zone disappeared as the great drought caused the flow of Mississippi to fall to its lowest level since 1933. During times of heavy flooding in the Mississippi River Basin, as in 1993, ""the "dead zone" dramatically increased in size, approximately 5,000 km (3,107 mi) larger than the previous year".

Locations

In the 1970s, marine dead zones were first noted in settled areas where intensive economic use stimulated scientific scrutiny: in the U.S. East Coast's Chesapeake Bay, in Scandinavia's strait called the Kattegat, which is the mouth of the Baltic Sea and in other important Baltic Sea fishing grounds, in the Black Sea, and in the northern Adriatic.

Other marine dead zones have appeared in coastal waters of South America, China, Japan, and New Zealand.

A 2008 study counted 405 dead zones worldwide.

Baltic Sea

Elizabeth River, Virginia

Lake Erie

Lower St. Lawrence Estuary

Oregon

Gulf of Mexico 'dead zone'

Causes

Dead zones are often caused by the decayof algae during algal blooms, like this one off the coast of La Jolla, San Diego, California.

Climate has a significant impact on the growth and decline of ecological dead zones. During spring months, as rainfall increases, more nutrient-rich water flows down the mouth of the Mississippi River. At the same time, as sunlight increases during the spring, algal growth in the dead zones increases dramatically. In fall months, tropical storms begin to enter the Gulf of Mexico and break up the dead zones, and the cycle repeats again in the spring.

Aquatic and marine dead zones can be caused by an increase in chemical nutrients (particularly nitrogen and phosphorus) in the water, known as eutrophication. These chemicals are the fundamental building blocks of single-celled, plant-like organisms that live in the water column, and whose growth is limited in part by the availability of these materials. Eutrophication can lead to rapid increases in the density of certain types of these phytoplankton, a phenomenon known as an algal bloom.

Limnologist Dr. David Schindler, whose research at the Experimental Lakes Area led to the banning of harmful phosphates in detergents, warned about algal blooms and dead zones,

"The fish-killing blooms that devastated the Great Lakes in the 1960s and 1970s haven't gone away; they've moved west into an arid world in which people, industry, and agriculture are increasingly taxing the quality of what little freshwater there is to be had here....This isn't just a prairie problem. Global expansion of dead zones caused by algal blooms is rising rapidly."

The major groups of algae are Cyanobacteria, green algae, Dinoflagellates, Coccolithophores and Diatom algae. Increase in input of nitrogen and phosphorus generally causes Cyanobacteria to bloom. Cyanobacteria are not good food for zooplankton and fish and hence accumulate in water, die, and then decompose. The bacterial degradation of their biomass consumes the oxygen in the water, thereby creating the state of hypoxia. Other algae are consumed and hence do not accumulate to the same extent as Cyanobacteria. Dead zones can be caused by natural and by anthropogenic factors. Natural causes include coastal upwelling and changes in wind and water circulation patterns. Use of chemical fertilizers is considered the major human-related cause of dead zones around the world. Runoff from sewage, urban land use, and fertilizers can also contribute to eutrophication.

Notable dead zones in the United States include the northern Gulf of Mexico region, surrounding the outfall of the Mississippi River, the coastal regions of the Pacific Northwest, and the Elizabeth River in Virginia Beach, all of which have been shown to be recurring events over the last several years.

Additionally, natural oceanographic phenomena can cause deoxygenation of parts of the water column. For example, enclosed bodies of water, such as fjords or the Black Sea, have shallow sills at their

entrances causing water to be stagnant there for a long time. The eastern tropical Pacific Ocean and northern Indian Ocean have lowered oxygen concentrations which are thought to be in regions where there is minimal circulation to replace the oxygen that is consumed. These areas are also known as oxygen minimum zones (OMZ). In many cases, OMZs are permanent or semipermanent areas.

Remains of organisms found within sediment layers near the mouth of the Mississippi River indicate four hypoxic events before the advent of artificial fertilizer. In these sediment layers, anoxia-tolerant species are the most prevalent remains found. The periods indicated by the sediment record correspond to historic records of high river flow recorded by instruments at Vicksburg, Mississippi.

Changes in ocean circulation triggered by ongoing climate change could also add or magnify other causes of oxygen reductions in the ocean.

In August 2017, a report found that the US meat industry is responsible for the largest-ever dead zone in the Gulf of Mexico. Runoff from widespread manure and fertilizer pollution contaminated water from the Heartland to the Gulf. Much of this pollution comes from the vast quantities of corn and soy used to raise meat animals for agribusiness companies, like Tyson.

Effects

Underwater video frame of the sea floor in the western Baltic covered with dead or dying crabs, fish and clams killed by oxygen depletion

Low oxygen levels recorded along the Gulf Coast of North America have led to reproductive problems in fish involving decreased size of reproductive organs, low egg counts and lack of spawning.

In a study of the Gulf killifish by the Southeastern Louisiana University done in three bays along the Gulf Coast, fish living in bays where the oxygen levels in the water dropped to 1 to 2 parts per million (ppm) for three or more hours per day were found to have smaller reproductive organs. The male gonads were 34% to 50% as large as males of similar size in bays where the oxygen levels were normal (6 to 8 ppm). Females were found to have ovaries that were half as large as those in normal oxygen levels. The number of eggs in females living in hypoxic waters were only one-seventh the number of eggs in fish living in normal oxygen levels.

Fish raised in laboratory-created hypoxic conditions showed extremely low sex hormone concentrations and increased elevation of activity in two genes triggered by the hypoxia-inductile factor (HIF) protein. Under hypoxic conditions, HIF pairs with another protein, ARNT. The two then bind to DNA in cells, activating genes in those plant cells.

Under normal oxygen conditions, ARNT combines with estrogen to activate genes. Hypoxic cells *in vitro* did not react to estrogen placed in the tube. HIF appears to render ARNT unavailable to interact with estrogen, providing a mechanism by which hypoxic conditions alter reproduction in fish.

It might be expected that fish would flee the potential suffocation, but they are often quickly rendered unconscious and doomed. Slow moving bottom-dwelling creatures like clams, lobsters and oysters are unable to escape. All colonial animals are extinguished. The normal re-mineralization and recycling that occurs among benthic life-forms is stifled.

It has been shown that future changes in oxygen could affect most marine ecosystems and have socio-economic ramifications due to human dependency on marine goods and services.

Coral reefs

History

The times of maximum reef development were in the Middle Cambrian (513–501 Ma), Devonian (416–359 Ma) and Carboniferous (359–299 Ma), owing to order Rugosa extinct corals and Late Cretaceous(100–66 Ma) and all Neogene (23 Ma–present), owing to order Scleractinia corals.

Not all reefs in the past were formed by corals: those in the Early Cambrian (542–513 Ma) resulted from calcareous algae and archaeocyathids (small animals with conical shape, probably related to sponges) and in the Late Cretaceous (100–66 Ma), when reefs formed by a group of bivalves called rudistsexisted; one of the valves formed the main conical structure and the other, much smaller valve acted as a cap.

Measurements of the oxygen isotopic composition of the aragonitic skeleton of coral reefs, such as *Porites*, can indicate changes in sea surface temperature and sea surface salinity conditions during the growth of the coral. This technique is often used by climate scientists to infer a region's paleoclimate.

Biodiversity

Over 4,000 species of fish inhabit coral reefs.

Organisms can cover every square inch of a coral reef.

Coral reefs form some of the world's most productive ecosystems, providing complex and varied marine habitats that support a wide range of other organisms. Fringing reefs just below low tide level have a mutually beneficial relationship with mangrove forests at high tide level and sea grass meadows in between: the reefs protect the mangroves and seagrass from strong currents and waves that would damage them or erode the sediments in which they are rooted, while the mangroves and sea grass protect the coral from large influxes of silt, fresh water and pollutants. This level of variety in the environment benefits many coral reef animals, which, for example, may feed in the sea grass and use the reefs for protection or breeding.

Reefs are home to a variety of animals, including fish, seabirds, sponges, cnidarians (which includes some types of corals and jellyfish), worms, crustaceans (including shrimp, cleaner shrimp, spiny lobsters and crabs), mollusks (including cephalopods), echinoderms (including starfish, sea urchins and sea cucumbers), sea squirts, sea turtles and sea snakes. Aside from humans, mammals are rare on coral reefs, with visiting cetaceans such as dolphins the main exception. A few species feed directly on corals, while others graze on algae on the reef. Reef biomass is positively related to species diversity.

The same hideouts in a reef may be regularly inhabited by different species at different times of day.

Nighttime predators such as cardinalfish and squirrelfish hide during the day, while damselfish, surgeonfish, triggerfish, wrasses and parrotfish hide from eels and sharks.

Algae

Reefs are chronically at risk of algal encroachment. Overfishing and excess nutrient supply from onshore can enable algae to outcompete and kill the coral. Increased nutrient levels can be a result of sewage or chemical fertilizer runoff. Runoff can carry nitrogen and phosphorus which promote excess algae growth. Algae can sometimes out-compete the coral for space. The algae can then smother the coral by decreasing the oxygen supply available to the reef. Decreased oxygen levels can slow down calcification rates, weakening the coral and leaving it more susceptible to disease and degradation Algae inhabit a large percentage of surveyed coral locations. The algal population consists of turf algae, coralline algae and macro algae. Some sea urchins (such as *Diadema antillarum*) eat these algae and could thus decrease the risk of algal encroachment.

Sponges

Sponges are essential for the functioning of the coral reef that system. Algae and corals in coral reefs produce organic material. This is filtered through sponges which convert this organic material into small particles which in turn are absorbed by algae and corals.

Fish

Over 4,000 species of fish inhabit coral reef The reasons for this diversity remain unclear. Hypotheses include the "lottery", in which the first (lucky winner) recruit to a territory is typically able to defend it against latecomers, "competition", in which adults compete for territory, and less-competitive species must be able to survive in poorer habitat, and "predation", in which population size is a function of postsettlement piscivore mortality. Healthy reefs can produce up to 35 tons of fish per square kilometer each year, but damaged reefs produce much less.

Invertebrates

Sea urchins, *Dotidae* and sea slugs eat seaweed. Some species of sea urchins, such as *Diadema antillarum*, can play a pivotal part in preventing algae from overrunning reefs. Researchers are investigating the use of native collector urchins, *Tripneustes gratilla*, for their potential as biocontrol agents to mitigate the spread of invasive algae species on coral reefs. *Nudibranchia* and sea anemones eat sponges.

A number of invertebrates, collectively called "cryptofauna," inhabit the coral skeletal substrate itself, either boring into the skeletons (through the process of bioerosion) or living in pre-existing voids and crevices. Animals boring into the rock include sponges, bivalve mollusks, and sipunculans. Those settling on the reef include many other species, particularly crustaceans and polychaete worms.

Seabirds

Coral reef systems provide important habitats for seabird species, some endangered. For example, Midway

Atoll in Hawaii supports nearly three million seabirds, including two-thirds (1.5 million) of the global population of Laysan albatross, and one-third of the global population of black-footed albatross. Each seabird species has specific sites on the atoll where they nest. Altogether, 17 species of seabirds live on Midway. The short-tailed albatross is the rarest, with fewer than 2,200 surviving after excessive feather hunting in the late 19th century.

Other

Sea snakes feed exclusively on fish and their eggs. Marine birds, such as herons, gannets, pelicans and boobies, feed on reef fish. Some land-based reptiles intermittently associate with reefs, such as monitor lizards, the marine crocodile and semiaquatic snakes, such as *Laticauda colubrina*. Sea turtles, particularly hawksbill sea turtles, feed on sponges.

Coral

When alive, corals are colonies of small animals embedded in calcium carbonate shells. Coral heads consist of accumulations of individual animals called polyps, arranged in diverse shapes. Polyps are usually tiny, but they can range in size from a pinhead to 12 inches (30 cm) across.

Reef-building or hermatypic corals live only in the photic zone (above 50 m), the depth to which sufficient sunlight penetrates the water.

Skeleton

Reefs grow as polyps and other organisms deposit calcium carbonate, the basis of coral, as a skeletal structure beneath and around themselves, pushing the coral head's top upwards and outwards. Waves, grazing fish (such as parrotfish), sea urchins, sponges and other forces and organisms act as bioeroders, breaking down coral skeletons into fragments that settle into spaces in the reef structure or form sandy bottoms in associated reef lagoons.

Typical shapes for coral species are named by their resemblance to terrestrial objects such as wrinkled brains, cabbages, table tops, antlers, wire strands and pillars. These shapes can depend on the life history of the coral, like light exposure and wave action, and events such as breakages.

Reproduction

Corals reproduce both sexually and asexually. An individual polyp uses both reproductive modes within its lifetime. Corals reproduce sexually by either internal or external fertilization. The reproductive cells are found on the mesenteries, membranes that radiate inward from the layer of tissue that lines the stomach cavity. Some mature adult corals are hermaphroditic; others are exclusively male or female. A few species change sex as they grow.

Internally fertilized eggs develop in the polyp for a period ranging from days to weeks. Subsequent development produces a tiny larva, known as a planula. Externally fertilized eggs develop during synchronized spawning. Polyps across a reef simultaneously release eggs and sperm into the water en masse. Spawn disperse over a large area. The timing of spawning depends on time of year, water temperature, and tidal and lunar cycles. Spawning is most successful given little variation between high and low tide. The less water movement, the better the chance for fertilization. Ideal timing occurs in the spring. Release of eggs or planula usually occurs at night, and is sometimes in phase with the lunar cycle (three to six days after a full moon). The period from release to settlement lasts only a few days, but some planulae can survive afloat for several weeks. They are vulnerable to predation and environmental conditions. The lucky few planulae that successfully attach to substrate then compete for food and space.

Locations

Boundary for 20 °C isotherms. Most corals live within this boundary. Note the cooler waters caused by upwelling on the southwest coast of Africa and off the coast of Peru.

Coral reefs are estimated to cover 284,300 km^2 (109,800 sq mi), just under 0.1% of the oceans' surface area. The Indo-Pacific region (including the Red Sea, Indian Ocean, Southeast Asia and the Pacific) account for 91.9% of this total. Southeast Asia accounts for 32.3% of that figure, while the Pacific including Australia accounts for 40.8%. Atlantic and Caribbean coral reefs account for 7.6%.

The Great Barrier Reef—largest, comprising over 2,900 individual reefs and 900 islands stretching for over 2,600 kilometers (1,600 mi) off Queensland, Australia

The Mesoamerican Barrier Reef System—second largest, stretching 1,000 kilometers (620 mi) from Isla Contoy at the tip of the Yucatán Peninsula down to the Bay Islands of Honduras

The New Caledonia Barrier Reef—second longest double barrier reef, covering 1,500 kilometers (930 mi)

The Andros, Bahamas Barrier Reef—third largest, following the east coast of Andros Island, Bahamas, between Andros and Nassau

The Red Sea—includes 6,000-year-old fringing reefs located along a 2,000 km (1,240 mi) coastline

The Florida Reef Tract—largest continental US reef and the third largest coral barrier reef, extends from Soldier Key, located in Biscayne Bay, to the Dry Tortugas in the Gulf of Mexico[51]

Pulley Ridge—deepest photosynthetic coral reef, Florida

Numerous reefs around the Maldives

The Philippines coral reef area, the second largest in Southeast Asia, is estimated at 26,000 square kilometers. 915 reef fish species and more than 400 scleractinian coral species, 12 of which are endemic are found there.

The Raja Ampat Islands in Indonesia's West Papua province offer the highest known marine diversity.

- Bermuda is known for its northernmost coral reef system, located at 32.4°N 64.8°W. The presence of coral reefs at this high latitude is due to the proximity of the Gulf Stream. Bermuda coral species represent a subset of those found in the greater Caribbean.

The world's northernmost individual coral reef is located within a bay of Japan's Tsushima Island in the Korea Strait.

The world's southernmost coral reef is at Lord Howe Island, in the Pacific Ocean off the east coast of Australia.

Formation

Most coral reefs were formed after the last glacial period when melting ice caused sea level to rise and flood continental shelves. Most coral reefs are less than 10,000 years old. As communities established themselves, the reefs grew upwards, pacing rising sea levels. Reefs that rose too slowly could become drowned, without sufficient light. Coral reefs are found in the deep sea away from continental shelves, around oceanic islands and atolls. The majority of these islands are volcanic in origin. Others have tectonic origins where plate movements lifted the deep ocean floor.

In *The Structure and Distribution of Coral Reefs*, Charles Darwin set out his theory of the formation

of atoll reefs, an idea he conceived during the voyage of the *Beagle*. He theorized that uplift and subsidence of the Earth's crust under the oceans formed the atolls.Darwin set out a

sequence of three stages in atoll formation. A fringing reef forms around an extinct volcanic island as the island and ocean floor subsides. As the subsidence continues, the fringing reef becomes a barrier reef and ultimately an atoll reef.

Darwin's theory starts with a volcanic islandwhich becomes extinct

As the island and ocean floor subside, coral growth builds a fringing reef, often including a shallow lagoon between the land and the main reef.

As the subsidence continues, the fringing reef becomes a larger barrier reef further from the shore with a bigger and deeper lagooninside.

Ultimately, the island sinks below the sea, and the barrier reef becomes an atoll enclosing an open lagoon.Darwin predicted that underneath each lagoon would be a bedrock base, the remains of the original volcano. Subsequent research supported this hypothesis. Darwin's theory followed from his understanding that coral polyps thrive in the tropics where the water is agitated, but can only live within a limited depth range, starting just below low tide. Where the level of the underlying earth allows, the corals grow around the coast to form fringing reefs, and can eventually grow become a barrier reef.

A fringing reef can take ten thousand years to form, and an atoll can take up to 30 million years.

Where the bottom is rising, fringing reefs can grow around the coast, but coral raised above sea level dies. If the land subsides slowly, the fringing reefs keep pace by growing upwards on a base of older, dead coral, forming a barrier reef enclosing a lagoon between the reef and the land. A barrier reef can encircle an island, and once the island sinks below sea level a roughly circular atoll of growing coral continues to keep up with the sea level, forming a central lagoon. Barrier reefs and atolls do not usually form complete circles, but are broken in places by storms. Like sea level rise, a rapidly subsiding bottom can overwhelm coral growth, killing the coral and the reef, due to what is called *coral drowning*. Corals that rely on zooxanthellae can die when the water becomes too deep for their symbionts to adequately photosynthesize, due to decreased light exposure.

The two main variables determining the geomorphology, or shape, of coral reefs are the nature of the substrate on which they rest, and the history of the change in sea level relative to that substrate.

The approximately 20,000-year-old Great Barrier Reef offers an example of how coral reefs formed on continental shelves. Sea level was then 120 m (390 ft) lower than in the 21st century. As sea level rose, the water and the corals encroached on what had been hills of the Australian coastal plain. By 13,000 years ago, sea level had risen to 60 m (200 ft) lower than at present, and many hills of the coastal plains had become continental islands. As sea level rise continued, water topped most of the continental islands. The corals could then overgrow the hills, forming cays and reefs. Sea level on the Great Barrier Reef has not changed significantly in the last 6,000 years. The age of living reef structure is estimated to be between 6,000 and 8,000 years. Although the Great Barrier Reef formed along a continental shelf, and not around a volcanic island, Darwin's principles apply. Development stopped at the barrier reef stage, since Australia is not about to submerge. It formed the world's largest barrier reef, 300–1,000 m (980–3,280 ft) from shore, stretching for 2,000 km (1,200 mi).

Healthy tropical coral reefs grow horizontally from 1 to 3 cm (0.39 to 1.18 in) per year, and grow vertically anywhere from 1 to 25 cm (0.39 to 9.84 in) per year; however, they grow only at depths shallower than 150 m (490 ft) because of their need for sunlight, and cannot grow above sea level.

Material

As the name implies, coral reefs are made up of coral skeletons from mostly intact coral colonies. As other chemical elements present in corals become incorporated into the calcium carbonate deposits, aragonite is formed. However, shell fragments and the remains of coralline algae such as the green-segmented genus *Halimeda* can add to the reef's ability to withstand damage from storms and other threats. Such mixtures are visible in structures such as Eniwetok Atoll.

Types

Since Darwin's identification of the three classical reef formations – the fringing reef around a volcanic island becoming a barrier reef and then an atoll – scientists have identified further reef types. While some sources find only three, Thomas and Goudie list four "principal large-scale coral reef types" – the fringing reef, barrier reef, atoll and table reef – while Spalding *et al.* list five "main types" – the fringing reef, barrier reef, atoll, "bank or platform reef" and patch reef.

Fringing reef

A fringing reef, also called a shore reef is directly attached to a shore, or borders it with an intervening narrow, shallow channel or lagoon. It is the most common reef type. Fringing reefs follow coastlines and can extend for many kilometres. They are usually less than 100 metres wide, but some hundreds of metres wide. Fringing reefs are initially formed on the shore at the low water level and expand seawards as they grow in size. The final width depends on where the sea bed begins to drop steeply. The surface of the fringe reef generally remains at the same height: just below the waterline. In older fringing reefs, whose outer regions pushed far out into the sea, the inner part is deepened by erosion and eventually forms a lagoon. Fringing reef lagoons can become over 100 metres wide and several metres deep. Like the fringing reef itself, they run parallel to the coast. The fringing reefs of the Red Sea are "some of the best developed in the world" and occur along all its shores except off sandy bays.

Barrier reef

Barrier reefs are separated from a mainland or island shore by a deep channel or lagoon. They resemble the later stages of a fringing reef with its lagoon, but differ from the latter mainly in size and origin. Their lagoons can be several kilometres wide and 30 to 70 metres deep. Above all, the offshore outer reef edge formed in open water rather than next to a shoreline. Like an atoll, it is thought that these reefs are formed either as the seabed lowered or sea level rose. Formation takes considerably longer than for a fringing reef, thus barrier reefs are much rarer.

The best known and largest example of a barrier reef is the Australian Great Barrier Reef. Other major examples are the Belize Barrier Reef and the New Caledonian Barrier Reef. Barrier reefs are also found on the coasts of Providencial, the Gambier Islands, on the southeast coast coast of Kalimantan, southeastern New Guinea and the south coast of the Louisiade Archipelago.

Platform reef

Platform reefs, variously called bank or table reefs, can form on the continental shelf, as well as in the open ocean, in fact anywhere where the seabed rises close enough to the surface of the ocean to enable the growth of zooxanthemic, reef-forming corals. Platform reefs are found in the southern Great Barrier Reef, the Swainand Capricorn Group on the continental shelf, about 100–200 km from the coast. Some platform reefs of the northern Mascarenes are several thousand kilometres from the mainland. Unlike fringing and barrier reefs which extend only seaward, platform reefs grow in all directions. They are variable in size, ranging from a few hundred metres to many kilometres across. Their usual shape is oval to elongated. Parts of these reefs can reach the surface and form sandbanks and small islands around which may form fringing reefs. A lagoon may form In the middle of a platform reef.

Platform reefs can be found within atolls. There they are called patch reefs and may reach only a few dozen metres in diameter. Where platform reefs form on an elongated structure, e. g. an old, eroded barrier reef, they can form a linear arrangement. This is the case, for example, on the east coast of the Red Sea near Jeddah. In old platform reefs, the inner part can be so heavily eroded that it forms a pseudo-atoll. These can be distinguished from real atolls only by detailed investigation, possibly including core drilling. Some platform reefs of the Laccadives are U-shaped, due to wind and water flow.

Atoll

Atolls or atoll reefs are a more or less circular or continuous barrier reef that extends all the way around a lagoon without a central island. They are usually formed from fringing reefs around volcanic islands.Over time, the island erodes away and sinks below sea level. Atolls may also be formed by the sinking of the seabed or rising of the sea level. A ring of reefs results, which enclose a lagoon. Atolls are numerous in the South Pacific, where they usually occur in mid-ocean, for example, in the Caroline Islands, the Cook Islands, French Polynesia, the Marshall Islands and Micronesia.

Atolls are found in the Indian Ocean, for example, in the Maldives, the Chagos Islands, the Seychelles and around Cocos Island. The entire Maldives consist of 26 atolls.

Other reef types or variants

Apron reef – short reef resembling a fringing reef, but more sloped; extending out and downward from a point or peninsular shore. The initial stage of a fringing reef.

Bank reef – isolated, flat-topped reef larger than a patch reef and usually on mid-shelf regions and linear or semi-circular in shape; a type of platform reef.

- **Patch reef** – common, isolated, comparatively small reef outcrop, usually within a lagoon or embayment, often circular and surrounded by sand or seagrass. Type of platform reef.

Ribbon reef – long, narrow, possibly winding reef, usually associated with an atoll lagoon. Also called a shelf-edge reef or sill reef.

Habili – reef specific to the Red Sea; does not reach near enough to the surface to cause visible surf; may be a hazard to ships (from the Arabic for "unborn")

Microatoll – community of species of corals; vertical growth limited by average tidal height; growth morphologies offer a low-resolution record of patterns of sea level change; fossilized remains can be dated using radioactive carbon dating and have been used to reconstruct Holocene sea levels

Cays – small, low-elevation, sandy islands formed on the surface of coral reefs from eroded material that piles up, forming an area above sea level; can be stabilized by plants to become habitable; occur in tropical environments throughout the Pacific, Atlantic and Indian Oceans (including the Caribbean and on the Great Barrier Reef and Belize Barrier Reef), where they provide habitable and agricultural land

Seamount or **guyot** – formed when a coral reef on a volcanic island subsides; tops of seamounts are rounded and guyots are flat; flat tops of guyots, or *tablemounts*, are due to erosion by waves, winds, and atmospheric processes

Mangroves

A **mangrove** is a shrub or small tree that grows in coastal saline or brackish water. The term is also used for tropical coastal vegetation consisting of such species. Mangroves occur worldwide in the tropics and subtropics, mainly between latitudes 25° N and 25° S. The total mangrove forest area of the world in 2000 was 137,800 square kilometres (53,200 sq mi), spanning 118 countries and territories.

Mangroves are salt-tolerant trees, also called halophytes, and are adapted to life in harsh coastal conditions. They contain a complex salt filtration system and complex root system to cope with salt water immersion and wave action. They are adapted to the low oxygen (anoxic) conditions of waterlogged mud.

The word is used in at least three senses: (1) most broadly to refer to the habitat and entire plant assemblage or *mangal*, for which the terms *mangrove forest biome*, and *mangrove swamp* are also used, (2) to refer to all trees and large shrubs in the mangrove swamp, and (3) narrowly to refer to the mangrove familyof plants, the Rhizophoraceae, or even more specifically just to mangrove trees of thegenus *Rhizophora*.

The mangrove biome, or mangal, is a distinct saline woodland or shrubland habitat characterized by depositional coastal environments, where fine sediments (often with high organic content) collect in areas protected from high-energy wave action. The saline conditions tolerated by various mangrove species range from brackish water, through pure seawater (3 to 4%), to water concentrated by evaporation to over twice the salinity of ocean seawater (up to 9%).

Etymology

The term "mangrove" comes to English from Spanish (perhaps by way of Portuguese), and is likely to originate from Guarani. It was earlier "mangrow" (from Portuguese *mangue* or Spanish *mangle*), but this word was corrupted via folk etymology influence of the word "grove".

Ecology

Mangrove swamps (mangals) are found in tropical and subtropical tidal areas. Areas where mangals occur include estuaries and marine shorelines.

The intertidal existence to which these trees are adapted represents the major limitation to the number of species able to thrive in their habitat. High tide brings in salt water, and when the tide recedes, solar evaporation of the seawater in the soil leads to further increases in salinity. The return of tide can flush out these soils, bringing them back to salinity levels comparable to that of seawater.

Mangrove plants require a number of physiological adaptations to overcome the problems of anoxia, high salinity and frequent tidal inundation. Each species has its own solutions to these problems; this may be the primary reason why, on some shorelines, mangrove tree species show distinct zonation. Small environmental variations within a mangal may lead to greatly differing methods for coping with the environment. Therefore, the mix of species is partly determined by the tolerances of individual species to physical conditions, such as tidal inundation and salinity, but may also be influenced by other factors, such as predation of plant seedlings by crabs.

Once established, mangrove roots provide an oyster habitat and slow water flow, thereby enhancing sediment deposition in areas where it is already occurring. The fine, anoxic sediments under mangroves act as sinks for a variety of heavy (trace) metals which colloidal particles in the sediments have scavenged from the water. Mangrove removal disturbs these underlying sediments, often creating problems of trace metal contamination of seawater and biota. Because of the uniqueness of mangrove ecosystems and the protection against erosion they provide, they are often the object of conservation programs, including national biodiversity action plans.

The unique ecosystem found in the intricate mesh of mangrove roots offers a quiet marine region for young organisms. In areas where roots are permanently submerged, the organisms they host include algae, barnacles, oysters, sponges, and bryozoans, which all require a hard surface for anchoring while they filter feed. Shrimps and mud lobsters use the muddy bottoms as their home. Mangrove crabs munch on the mangrove leaves, adding nutrients to the mangal muds for other bottom feeders. In at least some cases, export of carbon fixed in mangroves is important in coastal food webs.

Mangrove plantations in Vietnam, Thailand, Philippines and India host several commercially important species of fishes and crustaceans. Despite restoration efforts, developers and others have removed over half of the world's mangroves in recent times.

Mangrove forests can decay into peat deposits because of fungal and bacterial processes as well as by the action of termites. It becomes peat in good geochemical, sedimentary and tectonic conditions. The nature of these deposits depends on the environment and the types of mangrove involved. In Puerto Rico the red (Rhizophora mangle), white (Laguncularia racemosa) and black (Avicennia germinans) mangroves occupy different ecological niches and have slightly different chemical compositions so the carbon content varies between the species as well between the different tissues of the plant e.g. leaf matter vs roots.

Biology

Of the recognized 110 mangrove species, only about 54 species in 20 genera from 16 families constitute the "true mangroves", species that occur almost exclusively in mangrove habitats. Demonstrating convergent evolution, many of these species found similar solutions to the tropical conditions of variable salinity, tidal range (inundation), anaerobic soils and intense sunlight. Plant biodiversity is generally low in a given mangroveThe greatest biodiversity occurs in the mangal of New Guinea, Indonesia and Malaysia.

Adaptations to low oxygen

Red mangroves, which can survive in the most inundated areas, prop themselves above the water level with stilt roots and can then absorb air through pores in their bark (lenticels). Black mangroves live on higher ground and make many pneumatophores (specialised root-like structures which stick up out of the soil like straws for breathing) which are also covered in lenticels.

These "breathing tubes" typically reach heights of up to 30 cm, and in some species, over 3 m. The four types of pneumatophores are stilt or prop type, snorkel or peg type, knee type, and ribbon or plank type. Knee and ribbon types may be combined with buttress roots at the base of the tree. The roots also contain wide aerenchyma to facilitate transport within the plants.

Limiting salt intake

Red mangroves exclude salt by having significantly impermeable roots which are highly suberised(impregnated with suberin), acting as an ultra-filtration mechanism to exclude sodium salts from the rest of the plant. Analysis of water inside mangroves has shown 90% to 97% of salt has been excluded at the roots. In a frequently cited concept that has become known as the "sacrificial leaf", salt which does accumulate in the shoot (sprout) then concentrates in old leaves, which

the plant then sheds. However, recent research suggests the older, yellowing leaves have no more measurable salt content than the other, greener leaves.Red mangroves can also store salt in cell vacuoles. As seen in the photograph on the right, white or grey mangroves can secrete salts directly; they have two salt glands at each leaf base (correlating with their name—they are covered in white salt crystals).

Limiting water loss

Because of the limited fresh water available in salty intertidal soils, mangroves limit the amount of water they lose through their leaves. They can restrict the opening of their stomata (pores on the leaf surfaces, which exchange carbon dioxide gas and water vapour during photosynthesis). They also vary the orientation of their leaves to avoid the harsh midday sun and so reduce evaporation from the leaves. Anthony Calfo, a noted aquarium author, observed anecdotally a red mangrove in captivity only grows if its leaves are misted with fresh water several times a week, simulating frequent tropical rainstorms.

Nutrient uptake

Because the soil is perpetually waterlogged, little free oxygen is available.

Anaerobic bacteria liberate nitrogengas, soluble ferrum (iron), inorganic phosphates, sulfides and methane, which make the soil much less nutritious. Pneumatophores (aerial roots) allow mangroves to absorb gases directly from the atmosphere, and other nutrients such as iron, from the inhospitable soil. Mangroves store gases directly inside the roots, processing them even when the roots are submerged during high tide.

Increasing survival of offspring

In this harsh environment, mangroves have evolved a special mechanism to help their offspring survive. Mangrove seeds are buoyant and are therefore suited to water dispersal. Unlike most plants, whose seeds germinate in soil, many mangroves (e.g. red mangrove) are viviparous, whose seeds germinate while still

attached to the parent tree. Once germinated, the seedling grows either within the fruit (e.g. *Aegialitis*, *Avicennia* and *Aegiceras*), or out through the fruit (e.g. *Rhizophora*, *Ceriops*, *Bruguiera* and *Nypa*) to form a propagule (a ready-to-go seedling) which can produce its own food via photosynthesis.

The mature propagule then drops into the water, which can transport it great distances. Propagules can survive desiccation and remain dormant for over a year before arriving in a suitable environment. Once a propagule is ready to root, its density changes so the elongated shape now floats vertically rather than horizontally. In this position, it is more likely to lodge in the mud and root. If it does not root, it can alter its density and drift again in search of more favorable conditions.

Taxonomy and evolution

The following listing (modified from Tomlinson, 1986) gives the number of species of mangroves in each listed plant genus and family. Mangrove environments in the Eastern Hemisphere harbor six times as many species of trees and shrubs as do mangroves in the New World. Genetic divergence of mangrove lineages from terrestrial relatives, in combination with fossil evidence, suggests mangrove diversity is limited by evolutionary transition into the stressful marine environment, and the number of mangrove lineages has increased steadily over the Tertiary with little global extinction.

Major components

Family	**Genus, number of species**	**Common name**
Acanthaceae, **Avicenniaceae** or **Verbenaceae** (family allocation disputed)	*Avicennia*, 9	Black mangrove
Combretaceae	*Conocarpus*, 1; *Laguncularia*, 1; *Lumnitzera*, 3	Buttonwood, white mangrove
Arecaceae	*Nypa*, 1	Mangrove palm
Rhizophoraceae	*Bruguiera*, 7; *Ceriops*, 5; *Kandelia*, 2; *Rhizophora*, 8	Red mangrove
Lythraceae	*Sonneratia*, 5	

		Mangrove apple

Minor components

Family	Genus, number of species
Acanthaceae	*Acanthus*, 2; *Bravaisia*, 2
Arecaceae	*Phoenix*, 1
Bombacaceae	*Camptostemon*, 2
Cyperaceae	*Fimbristylis*, 1
Euphorbiaceae	*Excoecaria*, 2
Lecythidaceae	*Barringtonia*, 6
Lythraceae	*Pemphis*, 2

Meliaceae	*Xylocarpus*, 2
Myrtaceae	*Osbornia*, 1

Pellicieraceae *Pelliciera*, 1

Plumbaginaceae *Aegialitis*, 2

Primulaceae *Aegiceras*, 2

Pteridaceae *Acrostichum*, 3

Rubiaceae *Scyphiphora*, 1

Sterculiaceae *Heritiera*, 3

Geographical regions

Mangroves can be found in over 118 countries and territories in the tropical and subtropical regions of the world. The largest percentage of mangroves is found between the 5° N and 5° S latitudes. Approximately 75% of world's mangroves are found in just 15 countries. Asia has the largest amount (42%) of the world's mangroves, followed by Africa (21%), North/Central America (15%), Oceania (12%) and South America (11%).

Top 20 mangrove habitat nations in 2014

Rank	**Country**	**Tree cover (km^2) in mangrove forests**	**Tree cover (km^2) in mangrove biome**

1	Indonesia	23,143	42,278
2	Brazil	7,663	17,287
3	Malaysia	4,691	7,616
4	Papua New Guinea	4,169	6,236
5	Australia	3,315	3,314
6	Mexico	2,985	6,036
7	Nigeria	2,653	6,908
8	Myanmar	2,508	3,783
9	Venezuela	2,401	7,516

10	Philippines	2,060	2,084
11	Thailand	1,876	3,936
12	Bangladesh	1,773	2,314
13	Colombia	1,672	6,236
14	Cuba	1,624	2,407
15	United States	1,553	1,554

16	Panama	1,323	2,673
17	Mozambique	1,223	2,658
18	Cameroon	1,113	1,323

19	Gabon	1,081	3,864
20	Ecuador	935	1,906

Bioluminescenes and Biopigments

Bioluminescence is the production and emission of light by a living organism. It is a form of chemiluminescence. Bioluminescence occurs widely in marine vertebrates and invertebrates, as well as in some fungi, microorganisms including some bioluminescent bacteria and terrestrial invertebrates such as fireflies. In some animals, the light is bacteriogenic, produced by symbiotic organisms such as *Vibrio* bacteria; in others, it is autogenic, produced by the animals themselves.

In a general sense, the principal chemical reaction in bioluminescence involves some light-emitting molecule and an enzyme, generally called the luciferin and the luciferase, respectively. Because these are generic names, the luciferins and luciferases are often distinguished by including the species or group, i.e. Firefly luciferin. In all characterized cases, the enzyme catalyzes the oxidation of the luciferin.

In some species, the luciferase requires other cofactors such as calcium or magnesium ions, and sometimes also the energy-carrying molecule adenosine triphosphate (ATP). In evolution, luciferins vary little: one in particular, coelenterazine, is found in eleven different animal (phyla), though in some of these, the animals obtain it through their diet. Conversely, luciferases vary widely between different species, and consequently bioluminescence has arisen over forty times in evolutionary history.

Both Aristotle and Pliny the Elder mentioned that damp wood sometimes gives off a glow and many centuries later Robert Boyle showed that oxygen was involved in the process, both in wood and in glow-worms. It was not until the late nineteenth century that bioluminescence was properly investigated. The phenomenon is widely distributed among animal groups, especially in marine environments where dinoflagellates cause phosphorescence in the surface layers of water. On land it occurs in fungi, bacteria and some groups of invertebrates, including insects.

The uses of bioluminescence by animals include counter-illumination camouflage, mimicry of other animals, for example to lure prey, and signalling to other individuals of the same species, such as to attract mates. In the laboratory, luciferase-based systems are used in genetic engineering and for biomedical research. Other researchers are investigating the possibility of using bioluminescent systems for street and decorative lighting, and a bioluminescent plant has been created.

Evolution

E. N. Harvey (1932) was among the first to propose how bioluminescence could have evolved. In this early paper, he suggested that proto-bioluminescence could have arisen from respiratory chain proteins that hold fluorescent groups. This hypothesis has since been disproven, but it did lead to considerable interest in the origins of the phenomenon. Today, the two prevailing hypotheses (both concerning marine bioluminescence) are the ones put forth by Seliger (1993) and Rees et al. (1998).

Seliger's theory identifies luciferase enzymes as the catalyst for the evolution of bioluminescent systems. It suggests that the original purpose of luciferases was as mixed-function oxygenases. As the early ancestors of many species moved into deeper and darker waters natural selection applied forces that favored the development of increased eye sensitivity and enhanced visual signals. If selection were to favor a mutation in the oxygenase enzyme required for the breakdown of pigment molecules (molecules often associated with spots used to attract a mate or distract a predator) it could have eventually resulted in external luminescence in tissues.

Rees et al. (1998) uses evidence gathered from the marine luciferin coelenterazine to suggest that selection acting on luciferins may have arisen from pressures to protect oceanic organisms from potentially deleterious reactive oxygen species (ROS) (e.g. H_2O_2 and O_2^-). The functional shift from antioxidation to bioluminescence probably occurred when the strength of selection for antioxidation defense decreased as early species moved further down the water column. At greater depths exposure to ROS is significantly lower, as is the endogenous production of ROS through metabolism.

While popular at first, Seliger's theory has been challenged, particularly on the biochemical and genetic evidence that Rees examines. What remains clear, however, is that bioluminescence has evolved independently at least 40 times. Bioluminescence in fish began at least by the Cretaceousperiod. About 1,500 fish species are known to be bioluminescent; the capability evolved independently at least 27 times. Of these 27 occasions, 17 involved the taking up of bioluminous bacteria from the surrounding water while in the others, the intrinsic light evolved through chemical synthesis. These fish have become surprisingly diverse in the deep ocean and control their light with the help of their nervous system, using it not just to lure prey or hide from predators, but also for communication.

Chemical mechanism

Protein structure of the luciferase of the firefly *Photinus pyralis*. The enzyme is a much larger molecule than luciferin.

Bioluminescence is a form of chemiluminescence where light energy is released by a chemical reaction. This reaction involves a light-emitting pigment, the luciferin, and a luciferase, the enzyme component. Because of the diversity of luciferin/luciferase combinations, there are very few commonalities in the

chemical mechanism. From currently studied systems, the only unifying mechanism is the role of molecular oxygen, though many examples have a concurrent release of carbon dioxide. For example, the firefly luciferin/luciferase reaction requires magnesium and ATP and produces carbon dioxide (CO_2), adenosine monophosphate (AMP) and pyrophosphate (PP) as waste products. Other cofactors may be required for the reaction, such as calcium (Ca^{2+}) for the photoprotein aequorin, or magnesium (Mg^{2+}) ions and ATP for the firefly luciferase. Generically, this reaction could be described as:

Coelenterazine is a luciferin found in many different marine phyla from comb jellies to vertebrates. Like all luciferins, it is oxidised to produce light.

Instead of a luciferase, the jellyfish *Aequorea victoria* makes use of another type of protein called a photoprotein, in this case specifically aequorin. When calcium ions are added, the rapid catalysis creates a brief flash quite unlike the prolonged glow produced by luciferase. In a second, much slower, step luciferin is regenerated from the oxidised (oxyluciferin) form, allowing it to recombine with aequorin, in readiness for a subsequent flash. Photoproteins are thus enzymes, but with unusual reaction kinetics Furthermore, some of the blue light released by aequorin in contact with calcium ions is absorbed by a green fluorescent protein, which in turn releases green light in a process called resonant energy transfer.

Overall, bioluminescence has arisen over forty times in evolutionary history. In evolution, luciferins tend to vary little: one in particular, coelenterazine, is the light emitting pigment for nine phyla (groups of very different organisms), including polycystine radiolaria, Cercozoa (Phaeodaria), protozoa, comb jellies, cnidaria including jellyfish and corals, crustaceans, molluscs, arrow worms and vertebrates (ray-finned fish). Not all these organisms synthesize coelenterazine: some of them obtain it through their diet. Conversely, luciferase enzymes vary widely and tend to be different in each species.

Distribution

Huge numbers of bioluminescent dinoflagellates creating phosphorescence in breaking waves

Bioluminescence occurs widely among animals, especially in the open sea, including fish, jellyfish, comb jellies, crustaceans, and cephalopod molluscs; in some fungi and bacteria; and in various terrestrial invertebrates including insects. About 76% of the main taxa of deep-sea animals produce light. Most marine light-emission is in the blue and green light spectrum. However, some loose-jawed fish emit red and infrared light, and the genus *Tomopteris* emits yellow light.

The most frequently encountered bioluminescent organisms may be the dinoflagellatespresent in the surface layers of the sea, which are responsible for the sparkling phosphorescence sometimes seen at night in disturbed water. At least eighteen genera exhibit luminosity A different effect is the thousands of square miles of the ocean which shine with the light produced by bioluminescent bacteria, known as mareel or the milky seas effect.

Non-marine bioluminescence is less widely distributed, the two best-known cases being in fireflies and glow worms. Other invertebrates including insect larvae, annelids and arachnids possess bioluminescent abilities. Some forms of bioluminescence are brighter (or exist only) at night, following a circadian rhythm.

Uses in nature

Bioluminescence has several functions in different taxa. Steven Haddock et al. (2010) list as more or less definite functions in marine organisms the following: defensive functions of startle, counterillumination (camouflage), misdirection (smoke screen), distractive body parts, burglar alarm (making predators easier for higher predators to see), and warning to deter settlers; offensive functions of lure, stun or confuse prey, illuminate prey, and mate attraction/recognition. It is much easier for researchers to detect that a species is able to produce light than to analyse the chemical mechanisms or to prove what function the light serves. In some cases the function is unknown, as with species in three families of earthworm (Oligochaeta), such as *Diplocardia longa* where the coelomic fluid produces light when the animal moves The following functions are reasonably well established in the named organisms.

Counterillumination camouflage

Principle of counterilluminationcamouflage in firefly squid, *Watasenia scintillans*. When seen from below by a predator, the bioluminescence helps to match the squid's brightness and colour to the sea surface above.

In many animals of the deep sea, including several squid species, bacterial bioluminescence is used for camouflage by counterillumination, in which the animal matches the overhead environmental light as seen from below. In these animals, photoreceptors control the illumination to match the brightness of the background. These light organs are usually separate from the tissue containing the bioluminescent bacteria. However, in one species, *Euprymna scolopes*, the bacteria are an integral component of the animal's light organ.

Attraction

A fungus gnat from New Zealand, *Arachnocampa luminosa*, lives in the predator-free environment of caves and its larvae emit bluish-green light. They dangle silken threads that glow and attract flying insects, and wind in their fishing-lines when prey becomes entangled. The bioluminescence of the larvae of another fungus gnat from North America which lives on streambanks and under overhangs has a similar function. *Orfelia fultoni* builds sticky little webs and emits light of a deep blue colour. It has an inbuilt biological clock and, even when kept in total darkness, turns its light on and off in a circadian rhythm.

Fireflies use light to attract mates. Two systems are involved according to species; in one, females emit light from their abdomens to attract males; in the other, flying males emit signals to which the sometimes sedentary females respond. Click beetles emit an orange light from the abdomen when flying and a green light from the thorax when they are disturbed or moving about on the ground. The former is probably a sexual attractant but the latter may be defensive. Larvae of the click beetle *Pyrophorus nyctophanus* live

in the surface layers of termite mounds in Brazil. They light up the mounds by emitting a bright greenish glow which attracts the flying insects on which they feed.

In the marine environment, use of luminescence for mate attraction is chiefly known among ostracods, small shrimplike crustaceans, especially in the family Cyprididae. Pheromones may be used for long-distance communication, with bioluminescence used at close range to enable mates to "home in". A polychaete worm, the Bermuda fireworm creates a brief display, a few nights after the full moon, when the female lights up to attract males.

Defence

Many cephalopods, including at least 70 genera of squid, are bioluminescent. Some squid and small crustaceans use bioluminescent chemical mixtures or bacterial slurries in the same way as many squid use ink. A cloud of luminescent material is expelled, distracting or repelling a potential predator, while the animal escapes to safety. The deep sea squid *Octopoteuthis deletron* may autotomise portions of its arms which are luminous and continue to twitch and flash, thus distracting a predator while the animal flees.

Dinoflagellates may use bioluminescence for defence against predators. They shine when they detect a predator, possibly making the predator itself more vulnerable by attracting the attention of predators from higher trophic levels. Grazing copepods release any phytoplankton cells that flash, unharmed; if they were eaten they would make the copepods glow, attracting predators, so the phytoplankton's bioluminescence is defensive. The problem of shining stomach contents is solved (and the explanation corroborated) in predatory deep-sea fishes: their stomachs have a black lining able to keep the light from any bioluminescent fish prey which they have swallowed from attracting larger predators.

The sea-firefly is a small crustacean living in sediment. At rest it emits a dull glow but when disturbed it darts away leaving a cloud of shimmering blue light to confuse the predator. During World War II it was gathered and dried for use by the Japanese military as a source of light during clandestine operations.

The larvae of railroad worms (*Phrixothrix*) have paired photic organs on each body segment, able to glow with green light; these are thought to have a defensive purpose. They also have organs on the head which produce red light; they are the only terrestrial organisms to emit light of this colour.

Warning

Aposematism is a widely used function of bioluminescence, providing a warning that the creature concerned is unpalatable. It is suggested that many firefly larvae glow to repel predators; millipedes glow for the same purpose. Some marine organisms are believed to emit light for a similar reason. These include scale worms, jellyfish and brittle stars but further research is needed to fully establish the function of the luminescence. Such a mechanism would be of particular advantage to soft-bodied cnidarians if they were able to deter predation in this way. The limpet *Latia neritoides* is the only known freshwater gastropod that emits light. It produces greenish luminescent mucus which may have an anti-predator function. The marine snail *Hinea brasiliana* uses flashes of light, probably to deter predators.

The blue-green light is emitted through the translucent shell, which functions as an efficient diffuser of light.

Communication

Pyrosoma, a colonial tunicate; each individual zooid in the colony flashes a blue-green light.

Communication in the form of quorum sensing plays a role in the regulation of luminescence in many species of bacteria. Small extracellularly secreted molecules stimulate the bacteria to turn on genes for light production when cell density, measured by concentration of the secreted molecules, is high.

Pyrosomes are colonial tunicates and each zooid has a pair of luminescent organs on either side of the inlet siphon. When stimulated by light, these turn on and off, causing rhythmic flashing. No neural pathway runs between the zooids, but each responds to the light produced by other individuals, and even to light from other nearby colonies. Communication by light emission between the zooids enables coordination of colony effort, for example in swimming where each zooid provides part of the propulsive force.

Some bioluminous bacteria infect nematodes that parasitize Lepidoptera larvae. When these caterpillars die, their luminosity may attract predators to the dead insect thus assisting in the dispersal of both bacteria and nematodes. A similar reason may account for the many species of fungi that emit light. Species in the genera *Armillaria*, *Mycena*, *Omphalotus*, *Panellus*, *Pleurotus* and others do this, emitting usually greenish light from the mycelium, cap and gills. This may attract night-flying insects and aid in spore dispersal, but other functions may also be involved.

Quantula striata is the only known bioluminescent terrestrial mollusc. Pulses of light are emitted from a gland near the front of the foot and may have a communicative function, although the adaptive significance is not fully understood.

Mimicry

A deep sea anglerfish, *Bufoceratias wedli*, showing the esca (lure)

Bioluminescence is used by a variety of animals to mimic other species. Many species of deep sea fish such as the anglerfish and dragonfish make use of aggressive mimicry to attract prey. They have an appendage on their heads called an esca that contains bioluminescent bacteria able to produce a long lasting glow which the fish can control. The glowing esca is dangled or waved about to lure small animals to within striking distance of the fish.

The cookiecutter shark uses bioluminescence to camouflage its underside by counterillumination, but a small patch near its pectoral fins remains dark, appearing as a small fish to large predatory fish like tuna and mackerel swimming beneath it. When such fish approach the lure, they are bitten by the shark.

Female *Photuris* fireflies sometimes mimic the light pattern of another firefly, *Photinus*, to attract its males as prey. In this way they obtain both food and the defensive chemicals named lucibufagins, which *Photuris*cannot synthesize.

South American giant cockroaches of the genus *Lucihormetica* were believed to be the first known example of defensive mimicry, emitting light in imitation of bioluminescent, poisonous click beetles. However, doubt has been cast on this assertion, and there is no conclusive evidence that the cockroaches are bioluminescent.

Flashing of photophores of black dragonfish, *Malacosteus niger*, showing red fluorescence

Illumination

While most marine bioluminescence is green to blue, some deep sea barbeled dragonfishes in the genera *Aristostomias*, *Pachystomias* and *Malacosteus* emit a red glow. This adaptation allows the fish to see red-pigmented prey, which are normally invisible in the deep ocean environment where red light has been filtered out by the water column

The black dragonfish (also called the northern stoplight loosejaw) *Malacosteus niger* is believed to be one of the only fish to produce a red glow. Its eyes, however, are insensitive to this wavelength; it has an additional retinal pigment which fluoresces blue-green when illuminated. This alerts the fish to the presence of its prey. The additional pigment is thought to be assimilated from chlorophyllderivatives found in the copepods which form part of its diet.

Biotechnology

Biology and medicine

Bioluminescent organisms are a target for many areas of research. Luciferase systems are widely used in genetic engineering as reporter genes, each producing a different colour by fluorescence, and for biomedical research using bioluminescence imaging. For example, the firefly luciferase gene was used as early as 1986 for research using transgenic tobacco plants. *Vibrio* bacteria symbiose with marine invertebrates such as the Hawaiian bobtail squid (*Euprymna scolopes*), are key experimental models for bioluminescence Bioluminescent activated destruction is an experimental cancer treatment See also optogenetics which involves the use of light to control cells in living tissue, typically neurons, that have been genetically modified to express light-sensitive ion channels, and also see biophoton, a photon of non-thermal origin in the visible and ultraviolet spectrum emitted from a biological system.

Light production

The structures of photophores, the light producing organs in bioluminescent organisms, are being investigated by industrial designers. Engineered bioluminescence could perhaps one day be used toreduce the need for street lighting, or for decorative purposes if it becomes possible to produce light that is both bright enough and can be sustained for long periods at a workable price. The gene that makes the tails of

fireflies glow has been added to mustard plants. The plants glow faintly for an hour when touched, but a sensitive camera is needed to see the glow.University of Wisconsin–Madison is researching the use of genetically engineered bioluminescent E. coli bacteria, for use as bioluminescent bacteria in a light bulb. In 2011, Philips launched a microbial system for ambience lighting in the home. An iGEM team from Cambridge (England) has started to address the problem that luciferin is consumed in the light-producing reaction by developing a genetic biotechnology part that codes for a luciferin regenerating enzyme from the North American firefly; this enzyme "helps to strengthen and sustain light output". In 2016, Glowee, a French company started selling bioluminescent lights, targeting shop fronts and municipal street signs as their main markets. France has a law that forbids retailers and offices from illuminating their windows between 1 and 7 in the morning in order to minimise energy consumption and pollution.Glowee hoped their product would get around this ban. They used bacteria called *Aliivibrio fischeri* which glow in the dark, but the maximum lifetime of their product was three days.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: IMSc MB COURSE NAME: MARINE MICROBIOLOGY

COURSE CODE: 18MBP105A UNIT: IV BATCH-2018-2020

Sl. No	QUESTIONS	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	The protoplast can be used to	modify genetic information	create plant hybrid	study plant viral infections	no alteration	modify genetic information
2	The cell wall of	gram-positive bacteria are thicker than gram-negative bacteria	gram-negative bacteria are thicker than gram-positive bacteria	both have same thickness but composition is different	no change	gram-positive bacteria are thicker than gram-negative bacteria
3	Peptidoglycan is also known	N-acetyl muramic acid	murein mucopeptide	N acetylglucosamine	mesodiaminopimetic acid	murein mucopeptide
4	Which is most likely to be exposed on the surface of a gram-negative bacterium?	Pore protein (porin)	Protein involved in energy generation	Lipoteichoic acid	Phospholipids	Pore protein (porin)
5	The last step in synthesis of peptidoglycan is	attachment of a peptide to muramic acid	attaching two amino acids to form a cross-link	attachment of a portion of peptidoglycan to a membrane lipid	binding of penicillin to a membrane protein	attaching two amino acids to form a cross-link
6	Cytoplasmic inclusions include	ribosomes	Flagella	pili	Cell wall	ribosomes
7	The cocci which forms a bunch and irregular pattern are	Staphylococci	Diplococcic	Tetracocci	Streptococci	Staphylococci
8	Chemotaxis is a phenomenon of	swimming away of bacteria	swimming towards bacteria	swimming away or towards of bacteria in	no swim	swimming away or towards of bacteria in presence of

				presence of chemical compound		chemical compound
9	The structure responsible for transformation of bacteria is	pilli	Flagella	sheath	capsules	pilli
10	The next to last step in peptidoglycan biosynthesis is	synthesis of the NAM-peptide subunit	removal of the subunit from bactoprenol	linking the sugar of the disaccharide-peptide unit to the growing peptidoglycan chain	cross-linking the peptide side chains of peptidoglycan	cross-linking the peptide side chains of peptidoglycan
11	The cocci which forms a four is	Streptococci	Diplococcic	Staphylococci	Tetracocci	Tetracocci
12	The arrangement, in which flagella are distributed all round the bacterial cell, is known as	lophotrichous	Amphitrichous	peritrichous	monotrichous	Peritrichous
13	Periplasm is	the area between the inner and outer membranes of gram-negative bacteria	the area between the inner and outer membranes of Gram-positive bacteria	the interior portion of mitochondria	the area outside the cell membrane that is influenced by the polymers	the area between the inner and outer membranes of gram-negative bacteria
14	Which of the following has peptidoglycan as a major constituent of cell wall?	Gram-negative bacteria	Gram-positive bacteria	Fungi	virus	Gram-positive bacteria
15	The common word for bacteria	cooci	Pleomorphic	bacillus	spirilla	Spirilla

	which are helically curved rods is					
16	The bacteria deficient in cell wall is	Treponema	Mycoplasma	Staphylococcus	Klebsiella	Mycoplasma
17	Which of the following is not true about peptidoglycan?	It is a polymer consisting of N-acetyl glucosamine, N-acetyl muramic acid and amino acids (alanine, lysine, etc.)	It is present in prokaryotic cell wall	It occurs in the form of a bag shaped macro molecule surrounding the cytoplasm membrane	None of the above	It occurs in the form of a bag shaped macro molecule surrounding the cytoplasm membrane
18	The common word for bacteria which are irregular in shape is	cocci	bacilli	spirilla	pleomorphic	Pleomorphic
19	Single or clusters of flagella at both poles is known as	monotrichous	peritrichous	amphitrichous	atrichous	Amphitrichous
20	Which of the following bacterial genera (that produces endospore) have medical importance?	Shigella	Bacillus	vibrio	Coliform	Bacillus
21	Pharmacokinetics is:	The study of biological and therapeutic effects of drugs	The study of absorption, distribution, metabolism and excretion of drugs	The study of mechanisms of drug action	The study of methods of new drug development	The study of absorption, distribution, metabolism and excretion of drugs
22	The main mechanism of drugs absorption in GI tract :	Active transport (carrier-mediated	Filtration (aqueous	Endocytosis and exocytosis	Passive diffusion (lipid	Passive diffusion (lipid diffusion)

		diffusion)	diffusion)		diffusion)	
23	What does the term "bioavailability" mean?	Plasma protein binding degree of substance	Permeability through the brain-blood barrier	Fraction of an uncharged drug reaching the systemic circulation following any route administration	Amount of a substance in urine relative to the initial dose	Fraction of an uncharged drug reaching the systemic circulation following any route administration
24	Which route of drug administration is most likely to lead to the first-pass effect?	Sublingual	Oral	Intravenous	Intramuscular	Oral
25	The volume of distribution (Vd) relates:	Single to a daily dose of an administrated drug	An administrated dose to a body weight	An uncharged drug reaching the systemic circulation	The amount of a drug in the body to the concentration of a drug in plasma	The amount of a drug in the body to the concentration of a drug in plasma
26	Metabolic transformation (phase 1) is:	Acetylation and methylation of substances	Transformation of substances due to oxidation, reduction or hydrolysis	Glucuronide formation	Binding to plasma proteins	Transformation of substances due to oxidation, reduction or hydrolysis
27	Which organ involved in first pass effect?	Heart	Kidney	Brain	Liver	Liver

28	Which one of the following is not a route of administration?	Intravenous	Oral	Topical	Dissolution	Dissolution
29	Which of the following processes proceeds in the second phase of biotransformation?	Acetylation	Reduction	Oxidation	Hydrolysis	Acetylation
30	Which enzyme is involved in phase I metabolism	Catalase	Polyphenol oxidase	Cytochorome p450 MO	Oxygenase	Cytochorome p450 MO
31	Cytochorome p450 MO is found mainly in	Heart	Liver	Brain	Kidney	Liver
32	Dichloroisopropylarterenol blocks	Alpha adrenergic receptors	Beta adrenergic receptors	Both alpha and beta receptors	Gamma adrenergic receptors	Beta adrenergic receptors
33	Half life (t ½) is the time required to:	Change the amount of a drug in plasma by half during elimination	Metabolize a half of an introduced drug into the active metabolite	Absorb a half of an introduced drug	Bind a half of an introduced drug to plasma proteins	Change the amount of a drug in plasma by half during elimination
34	Irreversible interaction of an antagonist with a receptor is due to:	Ionic bonds	Hydrogen bonds	Covalent bonds	Sulphur bond	Covalent bonds
35	The second messenger of G-protein-coupled (metabotropic) receptor:	Adenylyl cyclase	Sodium ions	Phospholipase C	cAMP	cAMP
36	Give the definition for a therapeutical dose:	The amount of a substance to produce the minimal biological	The amount of a substance to produce effects	The amount of a substance to produce the	The amount of a substance to accelerate an	The amount of a substance to produce the required effect in

		effect	hazardous for an organism	required effect in most patients	increase of concentration of medicine in an organism	most patients
37	The substance which changes the activity of an effector element but doesn't belong to second messengers:	cAMP	cGMP	G–protein	Calcium ions	G–protein
38	An agonist can produce submaximal effects and has moderate efficacy it's called:	Partial agonist	Antagonist	Agonist-antagonist	Full agonist	Partial agonist
39	Conjugation is:	Process of drug reduction by special enzymes	Process of drug oxidation by special oxidases	Coupling of a drug with an endogenous substrate	Solubilization in lipids	Coupling of a drug with an endogenous substrate
40	What is implied by "active transport"?	Transport of drugs trough a membrane by means of diffusion	Transport without energy consumption	Engulf of drug by a cell membrane with a new vesicle formation	Transport against concentration gradient	Transport against concentration gradient
41	What kind of substances can't permeate membranes by passive diffusion?	Lipid-soluble	Non-ionized substances	Hydrophobic substances	Hydrophilic substances	Hydrophilic substances
42	The reasons determining bioavailability are:	Rheological parameters of blood	Amount of a substance obtained orally	Extent of absorption and hepatic first-pass	Glomerular filtration rate	Extent of absorption and hepatic first-pass effect

			and quantity of intakes	effect		
43	For the calculation of the volume of distribution (Vd) one must take into account:	Concentration of a substance in plasma	Concentration of substance in urine	Therapeutical width of drug action	A daily dose of drug	Concentration of a substance in plasma
44	Biotransformation of a medicinal substance results in:	Faster urinary excretion	Slower urinary excretion	Easier distribution in organism	Higher binding to membranes	Faster urinary excretion
45	The organelle that carry Cytochorome p450 MO is	Endoplasmic reticulam	Golgi complex	Mitochondria	Mitochondria	Endoplasmic reticulam
46	Conjugation of a drug includes the following EXCEPT:	Glucoronidation	Sulfate formation	Hydrolysis	Methylation	Hydrolysis
47	The phase II reaction which produce a compound with greater pharmocological actvity	Glucoronic acid conjugation	Conjugation with amino acid	Methylation	Glutathione conjugation	Methylation
48	Elimination is expressed as follows:	Rate of renal tubular reabsorption	Clearance speed of some volume of blood from substance	Time required to decrease the amount of drug in plasma by one-half	Clearance of an organism from a xenobiotic	Clearance of an organism from a xenobiotic
49	Acidic drug rapidly absorbed at	Stomach	GI tract	Large intestine	Mouth	Stomach
50	Coenzyme required by Cytochorome p450 MO is	NADH	NADPH	Lipoic acid	TPP	NADPH
51	Basic drugs are absorbed in	small intestine	stomach	Large intestine	Pancreas	small intestine
52	Which effect may lead to toxic	Refractoriness	Cumulative effect	Tolerance	Tachyphylaxis	Cumulative effect

	reactions when a drug is taken continuously or repeatedly?					
53	What term is used to describe a more gradual decrease in responsiveness to a drug, taking days or weeks to develop?	Refractoriness	Cumulative effect	Tolerance	Tachyphylaxis	Tolerance
54	What term is used to describe a decrease in responsiveness to a drug which develops in a few minutes?	Refractoriness	Cumulative effect	Tolerance	Tachyphylaxis	Tachyphylaxis
55	Which drug that cross the lipid bilayer easily	Water soluble drug	lipid soluble drug	ionsoluble drug	Non ionsoluble drug	lipid soluble drug
56	Science that deals with drug	Pharmacy	pharmacognosy	pharmacodynamics	pharmacology	Pharmacology
57	Inhibition of MAO causses an	decrease in the deamination of noradrenalin	increasse in the deamination of dopamine	increase in the deamination of noradrenalin	decrease in the deamination of dopamine	decrease in the deamination of noradrenalin
58	Systemic clearance (CLs) is related with:	Only the concentration of substances in plasma	Only the elimination rate constant	Volume of distribution, half life and elimination rate constant	Bioavailability and half life	Volume of distribution, half life and elimination rate constant
59	Elimination rate constant (Kelim) is defined by the following parameter:	Rate of absorption	Maximal concentration of a substance in	Highest single dose	Half life (t ½)	Half life (t ½)

			plasma			
60						

Unit V

Syllabus

Bar coding of marine organisms: Genome sequencing and physical mapping of genome. Marine exploration, Aquaculture-inland and freshwater, Isolation of marine bioactive compounds-separation, purification and identification techniques, cryopreservation.

DNA barcoding

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It differs from molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a preexisting classification. Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated, the utility of DNA barcoding for these purposes is subject to debate. The most commonly used barcode region, for animals, at least, is a segment of approximately 600 base pairs of the mitochondrial gene cytochrome oxidase I (COI).

Applications include, for example, identifying plant leaves even when flowers or fruit are not available, identifying insect larvae (which may have fewer diagnostic characters than adults and are frequently less well-known), identifying the diet of an animal, based on its stomach contents or faeces and identifying products in commerce (for example, herbal supplements, wood, or skins and other animal parts).

Choice of locus

A desirable locus for DNA barcoding should be standardized (so that large databases of sequences for that locus can be developed), present in most of the taxa of interest and sequenceable without species-specific PCR primers, short enough to be easily sequenced with current technology and provide a large variation between species yet a relatively small amount of variation within a species.

Although several loci have been suggested, a common set of standardized regions were selected by the respective committees:

For animals and many other eukaryotes, the mitochondrial COI gene

For plants, the concatenation of the rbcL and matK chloroplast genes. These provide poor resolution for land plants, and a call was made for regions to be assessed that could complement rbcL and matK.

For fungi, the internal transcribed spacer (ITS) region

Mitochondrial DNA

DNA barcoding is based on a relatively simple concept. All eukaryote cells contain mitochondria, and animal mitochondrial DNA (mtDNA) has a relatively fast mutation rate, resulting in the generation of diversity within and between populations over relatively short evolutionary timescales (thousands of generations). Typically, in animals, a single mtDNA genome is transmitted to offspring by each breeding female, and the genetic effective population size is proportional to the number of breeding females. This contrasts with the nuclear genome, which is around 100 000 times larger, where males and females each contribute two full genomes to the gene pool and effective size is

therefore proportional to twice the total population size. This reduction in effective population size leads to more rapid sorting of mtDNA gene lineages within and among populations through time, due to variance in fecundity among individuals (the principle of coalescence). The combined effect of higher mutation rates and more rapid sorting of variation usually results in divergence of mtDNA sequences among species and a comparatively small variance within species. A 658-bp region (the **Folmer region**) of the mitochondrialcytochrome c oxidase subunit I (COI) gene was proposed as a potential 'barcode.

(e.g., *Wolbachia*), as well as heteroplasmy, where an individual carries two or more mtDNA sequences, may affect patterns of mtDNA diversity within species, although these do not necessarily result in bar-coding failure. Occasional horizontal gene transfer
(such as via cellular symbionts, or other "reticulate"evolutionary phenomena in a lineage can lead to misleading results (i.e., it is possible for two different species

Additionally, some species may carry divergent mtDNA lineages segregating within populations, often due to historical geographic structure, where these divergent lineages do not reflect species boundaries. As of February 2013, the Barcode of Life Data Systems database included almost 2,000,000 barcode sequences from over 160,000 species of animals, plants, and fungi.

Identifying flowering plants

The use of the COI sequence "is not appropriate for most species of plants because of a much slower rate of cytochrome c oxidase I gene evolution in higher plants than in animals". A series of experiments was then conducted to find a more suitable region of the genome for use in the DNA barcoding of flowering plants (or the larger group of land plants). One 2005 proposal was the nuclear internal transcribed spacer region and the plastid trnH-psbA intergenic spacer; other researchers advocated other regions such as matK

In 2009, a collaboration of a large group of plant DNA barcode researchers proposed two chloroplast genes, rbcL and matK, taken together, as a barcode for plants. Adding the nuclear internal transcribed spacer ITS2 region was proposed to provide better resolution between species. As of 2015, the search for better DNA barcodes for plants continues, with the proposal that the chloroplast region *ycf1* may be suitable.

Artificial DNA

The use of artificial DNA sequences introduced into foodstuff offers an alternative application of DNA barcoding. While the read-out technologies stay the same, this approach enables the barcoding of non-natural properties, such as foodstuff manufacturer.

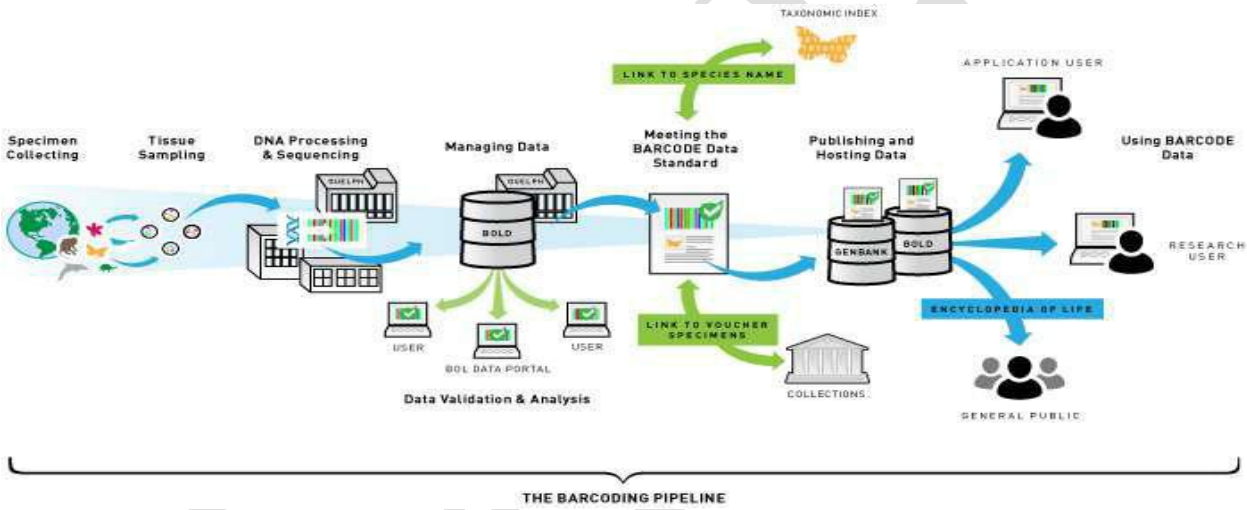
Vouchered specimens

DNA sequence databases like GenBank contain many sequences that are not tied to vouchered specimens (for example, herbarium specimens, cultured cell lines, or sometimes images). This is problematic in the face of taxonomic issues such as whether several species should be split or combined, or whether past identifications were sound. Therefore, best practice for DNA barcoding is to sequence vouchered specimens. Origin

The use of nucleotide sequence variations to investigate evolutionary relationships is not a new concept. Carl Woese used sequence differences in

ribosomal RNA (rRNA) to discover archaea, which in turn led to the redrawing of the evolutionary tree, and molecular markers (e.g., allozymes, rDNA, and mtDNA sequences) have been successfully used in molecular systematics for decades. DNA barcoding provides a standardised method for this process via the use of a short DNA sequence from a particular region of the genome to provide a 'barcode' for identifying species. In 2003, Paul D.N. Hebert from the University of Guelph, Ontario, Canada, proposed the compilation of a public library of DNA barcodes that would be linked to named specimens. This library would "provide a new master key for identifying species, one whose power will rise with increased taxon coverage and with faster, cheaper sequencing"

DNA Barcoding?



[In 2003, Paul Hebert, researcher at the University of Guelph in Ontario, Canada, proposed “DNA
barcoding” as a way to identify species. Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes productsusing the black stripes of the Universal Product Code (UPC). Two items may look very similar to the untrained eye, but in both cases the barcodes are distinct.

Until now, biological specimens were identified using morphological features like the shape, size and color of body parts. In some cases a trained technician could make routine identifications using morphological “keys” (step-by-step instructions of what to look for), but in most cases an experienced professional taxonomist is needed. If a specimen is damaged or is in an immature stage of development, even specialists may be unable to make identifications. Barcoding solves these problems because even non-specialists can obtain barcodes from tiny amounts of tissue. This is not to say that traditional taxonomy has become

less important. Rather, DNA barcoding can serve a dual purpose as a new tool in the taxonomists toolbox supplementing their knowledge as well as being an innovative device for non-experts who need to make a quick identification.

The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants.

Barcoding projects have four components:

The Specimens: Natural history museums, herbaria, zoos, aquaria, frozen tissue collections, seed banks, type culture collections and other repositories of biological materials are treasure troves of identified specimens.

The Laboratory Analysis: Laboratory protocols **(pdf;** 400Kb) can be followed to obtain DNA barcode sequences from these specimens. The best equipped molecular biology
labs can produce a DNA barcode sequence in a few hours. The data are then placed in a database for subsequent analysis.

The Database: One of the most important components of the Barcode Initiative is the construction of a public reference library of species identifiers which could be used to assign unknown specimens to known species. There are currently two main barcode databases that fill this role:

The International Nucleotide Sequence Database Collaborative is a partnership amongGenBank in the U.S., the Nucleotide Sequence Database of the European Molecular Biology Lab in Europe, and the DNA Data Bank of Japan. They have agreed to CBOL's data standards (pdf; 30Kb) for barcode records.

Barcode of Life Database (BOLD) was created and is maintained by University of Guelph in Ontario. It offers researchers a way to collect, manage, and analyze DNA barcode data.

The Data Analysis: Specimens are identified by finding the closest matching reference record in the database. CBOL's Data Analysis Working Group has created the Barcode of Life Data Portal which offers researchers new and more flexible ways to store, manage, analyze and display their barcode data.

The concept of DNA barcoding has become one of the most important and significant scientific visions in the last decade. As an emerging and effective

tool for species identification, the concept of DNA barcoding has gained worldwide popularity. The ground-breaking concept of DNA barcoding was put forward in the year 2003 by Professor Paul Hebert and collaborators serving at University of Guelph, Canada. Mitochondrial *cytochrome c oxidase* subunit 1 (COI) gene was suggested as unique barcode region for animals (Hebert et al., 2003). This sequence was validated at the 1st International Conference on DNA Barcode of Life. Henceforth, several studies have shown that the sequence diversity in a ~650 bp region near the 5′ region of the COI gene provides strong species level resolution for different animal groups like birds (Yoo et al., 2006, Tavares and Baker, 2008 and Schindel et al., 2011), springtails (Hogg and Hebert, 2004), shrimps (Trivedi et al., 2011), fishes (Ward et al., 2005, Yancy et al.,

2008,Bhattacharjee et al., 2012, Laskar et al., 2013 and Trivedi et al., 2014), tortoise (Kundu et al., 2013), oysters (Trivedi et al., 2012), mammals (Lim, 2012), spiders (Greenstone et al., 2005), mosquitoes (Cywinska et al., 2006), ticks (Zhang and Zhang, 2014) etc.

The Consortium for the Barcode of Life (CBOL) was established to support worldwide DNA barcoding and subsequently an international online data management system[7] – the Barcode of Life Data Systems (http://www.barcodinglife.org) came into effect. Survey and assessment of genetically diverse organisms of the earth through DNA barcoding is led by CBOL. A milestone in the field of DNA barcoding was achieved by launching of International Barcode of Life Project (iBOL). Canada was the first country to establish national network for DNA barcoding as The Canadian Barcode of Life Network (BOLNET.ca). Subsequently, several countries and regions have also established barcoding networks as part of the iBOL like Europe

(ECBOL; http://www.ecbol.org/), Norway (NorBOL; http://dnabarcoding.no/en/), Mexico (MexBOL; http://www.mexbol.org/) and Japan (JBOLI; http://www.jboli.org/). Besides this, thematic programs like human health (HealthBOL), polar life (PolarBOL) and quarantine and plant pathogens (QBOL, as a part of the ECBOL) are also in place.

Advantages of DNA barcoding in marine perspective

More than 70% of our planet is covered by oceans that have higher biodiversity compared to terrestrial or freshwater ecosystems. The massive marine ecosystem is the habitat for a large number of flora and fauna, both macro and micro. Among the 35 animal phyla, 34 phyla have marine representatives while 14 include exclusively marine animals (Briggs, 1994 and Gray, 1997). The

occurrence of cryptic species is relatively common in marine ecosystems. Cryptic species are those species that are
morphologically similar but genetically distinct. DNA barcoding can be a very effective tool in assessment of these cryptic species. Another problem that persists in the marine and estuarine habitat is the linking of the larval stages with the adult forms. DNA barcoding can accurately link the larval stages of a species in order to unravel the life cycle of different marine species, which is usually difficult and in some cases not possible using the morphological approach. The threat of invasive species to marine biodiversity can be globally assessed through DNA barcoding (Molnar et al., 2008).

The invasive alien species (IAS) poses severe threat and is capable of inflecting huge economic losses. DNA barcoding can be used to quickly and accurately identify the invasive alien species and prompt preventive measures with subsequent regulatory control can be initiated. Barcoding of indicator species can be fruitful in the monitoring

and abatement of marine pollution including coastal pollution. One main aim of DNA barcoding initiative is the discovery of new species. DNA barcoding can be used as an important tool for identification, authentication and safety assessment of sea food, particularly for processed, cooked or smoked products. This molecular identification can even allow us to trace the origin of certain products (Galimberti et al., 2013). A study conducted on the Japanese delicacy tuna sushi from different restaurants in USA, revealed the presence of endangered species, fraud and also a health hazard (Lowenstein et al., 2009). An analysis of 254 Canadian seafood samples revealed that 41% of the samples were mislabeled (Hanner et al., 2011).

DNA barcoding is an important tool in wildlife forensics and conservation. It can be used to identify endangered sea turtles by assessing turtle meat, carcasses or eggs that are illegally traded (Vargas et al., 2009). One important requirement of DNA barcoding is the collection and maintenance of samples as voucher specimens, which allows reliable means of corroborating the identification of the species from which data is accumulated. The voucher specimens provide permanent documentation for investigation of marine biodiversity. DNA barcoding has a great utility in the field of taxonomy (Ali et al., 2014).

DNA barcoding can be very effective for molecular phylogenic studies, geographical distribution and conservation of marine biodiversity. DNA barcoding can be used for pest

and disease control as well. With the recent developments in deep sea research and the revelation that several deep sea organisms possess extraordinary pharmaceutical properties, DNA barcoding of deep sea organisms has gained global attention. Census of the Diversity of Abyssal Marine Life (CeDAMar) is devoted to the barcoding of deep sea organisms. The user-friendliness of DNA barcodes is also an added advantage and can be effectively used for marine biodiversity assessment, fisheries management and conservation (Pérez1-Huete and Quezada, 2013).

Worldwide DNA barcoding initiative for marine organisms

MarBOL, the Marine Barcode of Life, is an international campaign to barcode marine species. MarBOL (http://www.marinebarcoding.org) is led by an International Steering Committee and an affiliated project of the Census of Marine Life (CoML). CoML is involved in several Ocean Realm Field Projects (Table 1). Already five International Barcode of Life Conferences have been held and the 6th International Barcode of Life

Conference is scheduled to be held in Guelph, Ontario, Canada during August 18–22, 2015.

Table 1. Involvement of Census of Marine Life (CoML) in various Ocean Realm Field Projects.

1	Arctic Ocean Diversity	ArcOD
2	Biogeography of Chemosynthetic Ecosystems	ChEss
3	Census of Antarctic Marine Life	CAML
4	Census of Diversity of Abyssal Marine Life	CeDAMar
5	Census of Marine Zooplankton	CMarZ
6	Continental Margin Ecosystems on a Worldwide Scale	CoMargE
7	Global Census of Coral Reef Ecosystems	CREEFS
8	Global Census of Marine Life on Seamounts	CenSeam
9	Gulf of Maine Area Program	GOMA

10	International Census of Marine Microbes	ICOMM
11	Natural Geography in Shore Areas	NaGISA
12	Pacific Ocean Shelf Tracking	POST
13	Tagging of Pacific Pelagics	TOPP

DNA barcoding of marine microbes

Assessment of biodiversity in the microbial world has always been a challenging task. Rapid and accurate identification of the microbes is frequently necessary to prevent the spread of diseases caused by microbes. Protists are eukaryotic microbes which have short generation time and asexual reproductive capability. An ecologically significant group of protists are the dinoflagellates which serve as primary producers, coral symbionts and cause red tides. DNA barcoding of marine environmental samples revealed massive dinoflagellate diversity

DNA barcoding of seagrasses, mangroves and marine phytoplanktons

Seagrasses are important submerged flowering plants that have very noticeable ecological influence on the coastal environment due to their nutrient recycling ability and high primary productivity. Besides this, they contain valuable secondary compounds like phenolic acids which are used in traditional medicines. Rosmarinic acid and zosteric acid obtained from seagrasses are widely used as an antioxidant and effective antifouling agent respectively. Although these marine plants have wide geographical distribution worldwide there is rapid decline in sea grass species and cover globally. It is reported that seagrasses are disappearing at the rate of 110 km per year, since 1980 Hence, there is urgent need for assessment and conservation of seagrasses.

Seagrasses perform both, sexual and asexual reproduction, but vegetative reproduction is more common and sexual progenies are short lived. Species identification becomes difficult because the flower as a distinct morphological trait is often unavailable. In such a situation, DNA barcoding can serve as a useful identification tool. Different markers have been used for identification of seagrasses like nuclear ITS for *Halophila* , trnK introns and rbcL for *Zostera* , ITS1, 5.8S rDNA and ITS2 for*Halophila*. By using rbcL and matK sequences it was revealed that it is possible to develop DNA barcoding for seagrasses

Mangroves at the intersection of terrestrial, estuarine and near shore marine ecosystem have immense ecological and economic significance. The ecosystem services provided by mangrove forests are worth at least US$1.6 billion per year worldwide. This dynamic and unique ecosystem is increasingly threatened and depleted. The conservation of mangroves is of utmost importance in order to maintain the health of this fragile environment. Loss of evolutionary unique species in the mangrove ecosystem has been reported and DNA barcoding provided phylogenetic information for developing unified mangrove management plan worldwide The Sunderbans is the single largest block of tidal halophytic mangrove forest listed in the UNESCO world heritage list (http://whc.unesco.org/en/list). It is regarded as the world's largest natural nursery where a large number of marine and estuarine species come to breed and the juveniles stay back to exploit its rich natural resources. In a study conducted in the Sunderbans mangrove ecosystem, molecular methods based on *rbc*L subunit of RuBisCO enzyme were used for identification of phytoplankton groups lesser than 10 µm size

DNA barcoding of marine algae

Different species of red marine macro algae are often difficult to identify by using morphological techniques. Two molecular markers namely mitochondrial COI gene and UPA (Universal Plastid Amplicon) domain V of the 23S rRNA gene were used for identification of different species of red alga belonging to the family Kallymeniaceae. Results showed that COI was a more sensitive marker and led to the discovery of a new species *Euthora timburtonii* (Clarkston and Saunders, 2010). A similar study was conducted involving inter tidal red macro algae in China with three molecular markers – COI, UPA and ITS (nuclear internal transcribed spacer). Although COI was effective to identify species but not all species gave successful amplicons due to lack of universal primers. UPA had effective universal primers but showed problems with closely related species, while ITS was the least effective.

Gracilariaceae is a red algal family which is commercially important for its use in biotechnology and microbiology research as a phycocolloid agar. *Gracilaria* species are difficult to identify morphologically and DNA barcoding holds promise in species level identification. Recently, a novel microalga was isolated and characterized from Indian Ocean which has biofuel potential. In this study 16S rRNA and 23S rRNA were used as barcode. DNA barcoding can be useful as a rapid, sensitive and reliable method for monitoring programs of marine and coastal ecosystems for detecting Harmful Algal Bloom (HAB) species.

DNA barcoding of marine zooplanktons

Zooplanktons have great ecological significance and represent 15 animal groups (phyla). Therefore, DNA barcoding of zooplanktons is an important aspect of modern ecological studies. Census for Marine Zooplanktons (CMarZ) is devoted to the study of global zooplankton assemblages. The DNA Barcoding Centers of CMarZ are located in UConn (USA), Bremerhaven (Germany), ORI (Japan), Qingdao (China) and Goa (India). Fig. 1shows the five CMarZ barcoding centers of the world. Barcode analysis using COI gene involving 52 specimens of 14 species of chaetognaths could successfully discriminate different species of chaetognaths across the phylum. The average K2P distance within species was 0.0145. Among the marine zooplanktons the copepods are one of the most systematically complex and ecologically significant groups with more than 2500 species. Several studies have been conducted on this diverse group. The occurrence of cryptic species is widespread among the copepods which necessitates more DNA barcoding studies. Some important publications on DNA barcoding of marine copepods are shown in Table 4.

Figure 1. Five CMarZ barcoding centers of the world.

Since it is difficult to identify the different chaetognath species based on morphological characters, especially with those preserved in alcohol, DNA barcoding can be very effective to resolve this problem. A study was conducted with*Neocalanus* copepods involving four marker genes namely COI, 12S, nuclear ITS, and 28S. The results showed that although all the four markers could identify distinctly all the species but distinction of the form variants was only confirmed by the COI sequences. DNA sequence variation of a 575 base-pair region of 28S rDNA, from North and South Atlantic regions could

accurately and reliably identify the three species of *Oithona*, an ecologically important copepod species.

DNA barcoding of marine invertebrates

The pteropods which belong to the phylum Mollusca and class Gastropoda are of unique research interest due to their vulnerability to ocean acidification. Barcoding of*Diacavolinia* pteropods indicated that the Atlantic specimens comprise a single monophyletic species and show probable species-level divergence between Atlantic and Pacific population. DNA barcoding comprising 227 species of Canadian marine mollusks

indicated possible cases of overlooked species. DNA barcoding projects should be developed for megadiverse groups such as mollusks to facilitate species discovery and conservation. A study involving 315 specimens from around 60 venerid species showed that DNA barcoding can be very effective in species delimitation. Marine oysters are bivalves that have great economic significance. Identification of oysters largely based on phenotypic characters like shell morphology is problematic due to the taxonomic controversies. Shell morphology, used as a primary distinguishing feature is greatly affected by habitat. In such cases, molecular identification proves to be useful (Table 4).

Echinoderms are exclusively marine animals. DNA barcoding of 191 echinoderm species belonging to five classes was undertaken. Based on shallow intraspecific versus deep congeneric divergences 97.9% specimens were assigned to known species. Sponges have canal system inside the body and possess pharmaceutical properties. Sponge Barcoding Project, http://www.spongebarcoding.org is a global initiative. A DNA barcoding workflow capable of analyzing large sponge collections has been developed through this project. Nematodes are known for their role as indicator of anthropogenic stress in the marine ecosystems. In the nematodes, 18S gene was able to amplify across several taxa and showed identification success rate of 97%. Universal primers for diverse group of marine metazoan invertebrates are available. (Table 4).

DNA barcoding of lower chordates

Ascidians are filter-feeding marine urochordates which are regarded as model organisms used to study complex biological processes. They are used to study the transcriptional control of embryonic development, mechanism of metal accumulation, evolution of the

immune system, conservation of gene regulatory networks in chordates, development of heart, etc. (Holland and Gibson-Brown, 2003, Trivedi et al., 2003, Satoh et al., 2003, Stolfi and Christiaen, 2012, Tolkin and Christiaen, 2012 and Razy-Krajka et al., 2014). The genome of an ascidian species *Ciona intestinalis* is the smallest of any experimentally manipulable chordate, as a consequence it is used in genome analysis studies. COI gene analysis of *Ciona* specimens from New Zealand revealed for the first time, the existence of solitary ascidian *Ciona savignyi* in the Southern Hemisphere (Smith et al., 2012). A new ascidian species belonging to the genus *Diplosoma* has been revealed through DNA barcoding in the Ryukyu Archipelago of Japan (Hirose and Hirose, 2009).

DNA barcoding of marine fishes

Marine fish is an important source of protein, vitamin D, vitamin B_{12}, iodine, selenium and omega-3 fatty acids. Marine fisheries sector has a very significant contribution in food security and economic welfare. Proper identification of fish species is important for management of fisheries and authentication of food products. DNA barcoding allows fast and efficient means of fish identification. Two main global barcoding initiatives for fish are FISH-BOL (http://www.fishbol.org) and SHARK-BOL (http://www.sharkbol.org). DNA barcoding is useful not only for the identification of whole fish but also for the identification of larvae, eggs, fillets, fins or other fragments of the body which are difficult to identify based on morphology. This molecular technique was used to identify shark fins that were confiscated from illegal fishers in Australia (Holmes et al., 2009). Demand for ornamental fish is rapidly increasing globally. COI gene analysis of 391

ornamental fish species from 8 coral reef locations revealed that most (98%) of these species belonged to distinct barcode clusters (Steinke et al., 2009a and Steinke et al., 2009b).

Whole genome sequencing

Whole genome sequencing (also known as **WGS**, **full genome sequencing**, **complete genome sequencing**, or **entire genome sequencing**) is the process of determining the complete DNA sequence of an organism's genome at a single time. This entails sequencing all of an organism's chromosomal DNA as well as DNA contained in the mitochondria and, for plants, in the chloroplast.

Whole genome sequencing has largely been used as a research tool, but is currently being introduced to clinics. [2][3][4] In the future of personalized medicine, whole genome sequence data will be an important tool to guide therapeutic intervention. [5] The tool

of gene sequencing at SNP level is also used to pinpoint functional variants from association studies and improve the knowledge available to researchers interested in evolutionary biology, and hence may lay the foundation for predicting disease susceptibility and drug response.

Whole genome sequencing should not be confused with DNA profiling, which only determines the likelihood that genetic material came from a particular individual or group, and does not contain additional information on genetic relationships, origin or susceptibility to specific diseases. In addition, whole genome sequencing should not be confused with methods that sequence specific subsets of the genome - such methods include whole exome sequencing (1% of the genome) or SNP genotyping (<0.1% of the genome). As of 2017 there were no complete genomes for any mammals, including humans.Between 4% to 9% of the human genome, mostly satellite DNA,
had not been sequenced.

Experimental details

Cells used for sequencing

Almost any biological sample containing a full copy of the DNA—even a very small amount of DNA or ancient DNA—can provide the genetic material necessary for full genome sequencing. Such samples may include saliva, epithelial cells, bone marrow, hair (as long as the hair contains a hair follicle), seeds, plant leaves, or anything else that has DNA-containing cells.

The genome sequence of a single cell selected from a mixed population of cells can be determined using techniques of *single cell genome sequencing*. This has important advantages in environmental microbiology in cases where a single cell of a particular microorganism species can be isolated from a mixed population by microscopy on the basis of its morphological or other distinguishing characteristics. In such cases the normally necessary steps of isolation and growth of the organism in culture may be
omitted, thus allowing the sequencing of a much greater spectrum of organism genomes.
Single cell genome sequencing is being tested as a method of preimplantation genetic diagnosis, wherein a cell from the embryo created by in vitro fertilization is taken and analyzed before embryo transfer into the uterus. After implantation, cell-free fetal DNA can be taken by simple venipuncturefrom the mother and used for whole genome sequencing of the fetus.

Early techniques

Sequencing of nearly an entire human genome was first accomplished in 2000 partly through the use of shotgun sequencing technology. While full genome shotgun sequencing for small (4000–7000 base pair) genomes was already in use in 1979, broader application benefited from pairwise end sequencing, known colloquially as *double-barrel shotgun sequencing*. As sequencing projects began to take on longer and more complicated genomes, multiple groups began to realize that useful information could be obtained by sequencing both ends of a

fragment of DNA. Although sequencing both ends of the same fragment and keeping track of the paired data was more cumbersome than sequencing a single end of two distinct fragments, the knowledge that the two sequences were oriented in opposite directions and were about the length of a fragment apart from each other was valuable in reconstructing the sequence of the original target fragment.

The first published description of the use of paired ends was in 1990 as part of the sequencing of the human HPRT locus, although the use of paired ends was limited to
closing gaps after the application of a traditional shotgun sequencing approach. The first theoretical description of a pure pairwise end sequencing strategy, assuming fragments of constant length, was in 1991. In 1995 the innovation of using fragments of varying sizes was introduced, and demonstrated that a pure pairwise end-sequencing strategy would be possible on large targets. The strategy was subsequently adopted by The Institute for Genomic Research (TIGR) to sequence the entire genome of the bacterium *Haemophilus influenzae* in 1995, and then by Celera Genomics to sequence the entire fruit fly genome in 2000, and subsequently the entire human genome. Applied Biosystems, now called Life Technologies, manufactured the automated capillary sequencers utilized by both Celera Genomics and The Human Genome Project.

Current techniques

While capillary sequencing was the first approach to successfully sequence a nearly full human genome, it is still too expensive and takes too long for commercial purposes. Since 2005 capillary sequencing has been progressively displaced by high-throughput (formerly "next-generation") sequencing technologies such as Illumina dye sequencing, pyrosequencing, and SMRT sequencing. All of these technologies continue to employ the basic shotgun strategy, namely, parallelization and template generation via genome fragmentation.

Other technologies are emerging, including nanopore technology. Though nanopore sequencing technology is still being refined, its portability and potential capability of generating long reads are of relevance to whole-genome sequencing applications.

Analysis

In principle, full genome sequencing can provide the raw nucleotide sequence of an individual organism's DNA. However, further analysis must be performed to provide the biological or medical meaning of this sequence, such as how this knowledge can be used to help prevent disease. Methods for analysing sequencing data are being developed and refined.

Because sequencing generates a lot of data (for example, there are approximately six billion base pairs in each human diploid genome), its output is stored electronically and requires a large amount of computing power and storage capacity.

While analysis of WGS data can be slow, it is possible to speed up this step by using dedicated hardware.

Physical Mapping

A map generated by genetic techniques is rarely sufficient for directing the sequencing phase of a genome project. This is for two reasons:

The resolution of a genetic map depends on the number of crossovers that have been scored . This is not a major problem for microorganisms because these can be obtained in huge numbers, enabling many crossovers to be studied, resulting in a highly detailed genetic map in which the markers are just a few kbapart. For example, when the *Escherichia coli* genome sequencing project began in 1990, the latest genetic map for this organism comprised over 1400 markers, an average of one per 3.3 kb. This was sufficiently detailed to direct the sequencing program without the need for extensive physical mapping. Similarly, the *Saccharomyces cerevisiae* project was supported by a fine-scale genetic map (approximately 1150 genetic markers, on average one per 10 kb). The problem with humans and most other eukaryotes is that it is simply not possible to obtain large numbers of progeny, so relatively few meioses can be studied and the resolving power of linkage analysis is restricted. This means that genes that are several tens of kb apart may appear at the same position on the genetic map.

Genetic maps have limited accuracy . We touched on this point when we assessed Sturtevant's assumption that crossovers occur at random along chromosomes. This assumption is only partly correct because the presence of recombination hotspots means that crossovers are more likely to occur at some points rather than at others. The effect that this can have on the accuracy of a genetic map was illustrated in 1992 when the complete sequence for *S. cerevisiae* chromosome III was published (Oliver *et al*., 1992), enabling the first direct comparison to be made between a genetic map and the actual positions of markers as shown by DNA sequencing. There were considerable

discrepancies, even to the extent that one pair of genes had been ordered incorrectly by genetic analysis. Bear in mind that *S. cerevisiae*is one of the two eukaryotes (fruit fly is the second) whose genomes have been subjected to intensive genetic mapping. If the yeast genetic map is inaccurate then how precise are the genetic maps of organisms subjected to less detailed analysis?

These two limitations of genetic mapping mean that for most eukaryotes a genetic map must be checked and supplemented by alternative mapping procedures before large-scale DNA sequencing begins. A plethora of physical mapping techniques has been developed to address this problem, the most important being:

Restriction mapping, which locates the relative positions on a DNA molecule of the recognition sequences for restriction endonucleases;

Fluorescent *in situ* hybridization (FISH), in which marker locations are mapped by hybridizing a probe containing the marker to intact chromosomes;

Sequence tagged site (STS) mapping, in which the positions of short sequences are mapped by PCR and/or hybridization analysis of genome fragments.

Restriction mapping

Genetic mapping using RFLPs as DNA markers can locate the positions of polymorphic restriction sites within a genome but very few of the restriction sites in a genome are polymorphic, so many sites are not mapped by this technique. Could we increase the marker density on a genome map by using an alternative method to locate the positions of some of the non-polymorphic restriction sites? This is what restriction mapping achieves, although in practice the technique has limitations which mean that it is applicable only to relatively small DNA molecules. We will look first at the technique and then consider its relevance to genome mapping.

Not all restriction sites are polymorphic.

The basic methodology for restriction mapping

The simplest way to construct a restriction map is to compare the fragment sizes produced when a DNA molecule is digested with two different restriction enzymes that recognize different target sequences. An example using the restriction enzymes *Eco*RI and *Bam*HI is shown in Figure 5.24. First, the DNA molecule is digested with just one of the enzymes and the sizes of the resulting fragments are measured by agarose gel electrophoresis. Next, the molecule is digested with the second enzyme and the resulting fragments again sized in an agarose gel. The results so far enable the number of restriction sites for each enzyme to be worked out, but do not allow their relative positions to be determined. Additional information is therefore obtained by cutting the DNA molecule with both enzymes together. In the example shown in Figure 5.24, this double restriction enables three of the sites to be mapped. However, a problem arises with the larger *Eco*RI fragment because this contains two *Bam*HI sites and there are two alternative possibilities for the map location of the outer one of these. The problem is solved by going back to the original DNA molecule and treating it again with *Bam*HI on its own, but this time preventing the digestion

from going to completion by, for example, incubating the reaction for only a short time or using a suboptimal incubation temperature. This is called a partial restriction and leads to a more complex set of products, the complete restriction products now being supplemented with partially restricted fragments that still contain one or more uncut *Bam*HI sites. In the example shown in, the size of one of the partial restriction fragments is diagnostic and the correct map can be identified.

Restriction mapping. The objective is to map the *Eco*RI (E) and *Bam*HI (B) sites in a linear DNA molecule of 4.9 kb. The results of single and double restrictions are shown at the top. The sizes of the fragments given after double restriction enable two (more...)

A partial restriction usually gives the information needed to complete a map, but if there are many restriction sites then this type of analysis becomes unwieldy, simply because there are so many different fragments to consider. An alternative strategy is simpler because it enables the majority of the fragments to be ignored. This is achieved by attaching a radioactive or other type of marker to each end of the starting DNA molecule before carrying out the partial digestion. The result is that many of the partial restriction products become 'invisible' because they do not contain an end-fragment and so do not show up when the agarose gel is screened for labeled products. The sizes of the partial restriction products that are visible enable unmapped sites to be positioned relative to the ends of the starting molecule.

The scale of restriction mapping is limited by the sizes of the restriction fragments

Restriction maps are easy to generate if there are relatively few cut sites for the enzymes being used. However, as the number of cut sites increases, so also do the numbers of single, double and partial restriction products whose sizes must be determined and compared in order for the map to be constructed. Computer analysis can be brought into play but problems still eventually arise. A stage will be reached when a digest contains so many fragments that individual bands merge on the agarose gel, increasing the chances of one or more fragments being measured incorrectly or missed out entirely. If several fragments have similar sizes then even if they can all be identified, it may not be possible to assemble them into an unambiguous map.

Restriction mapping is therefore more applicable to small rather than large molecules, with the upper limit for the technique depending on the frequency of the restriction sites in the molecule being mapped. In practice, if a DNAmolecule is less than 50 kb in length it is usually possible to construct an unambiguous restriction map for a selection of enzymes with six-nucleotide recognition sequences. Fifty kb is of course way below the minimum size for bacterial or eukaryotic chromosomes, although it does cover a few viral and organelle genomes, and whole -genome restriction maps have indeed been

important in directing sequencing projects with these small molecules. Restriction maps are equally useful after bacterial or eukaryotic genomic DNA has been cloned, if the cloned fragments are less than 50 kb, because a detailed restriction map can then be built up as a preliminary to sequencing the cloned region. This is an important application of restriction mapping in sequencing projects with large genomes, but is there any possibility of using restriction analysis for the more general mapping of entire genomes larger than 50 kb?

The answer is a qualified 'yes', because the limitations of restriction mapping can be eased slightly by choosing enzymes expected to have infrequent cut sites in the target DNA molecule. These 'rare cutters' fall into two categories:

Enzymes with seven- or eight-nucleotide recognition sequences . A few restriction enzymes cut at seven- or eight-nucleotide recognition sequences. Examples are *Sap*I (5′-GCTCTTC-3′) and *Sgf*I (5′-GCGATCGC-3′). The seven-nucleotide enzymes would be expected, on average, to cut a DNA molecule with a GC content of 50% once every 4^7 = 16 384 bp. The eight-nucleotide enzymes should cut once every 4^8 = 65 536 bp. These figures compare with 4^6 = 4096 bp for six-nucleotide enzymes such as *Bam*HI and *Eco*RI. Seven- and eight-nucleotide cutters are often used in restriction mapping of large molecules but the

approach is not as useful as it might be simply because not many of these enzymes are known.

Enzymes whose recognition sequences contain motifs that are rare in the target DNA . Genomic DNA molecules do not have random sequences and some are significantly deficient in certain motifs. For example, the sequence 5′-CG-3′ is rare in human DNA because human cells possess an enzyme that adds a methyl group to carbon 5 of the C nucleotide in this sequence. The resulting 5-methylcytosine is unstable and tends to undergo deamination to give thymine. The consequence is that during human evolution many of the 5′-CG-3′ sequences that were originally in our genome have become converted to 5′-TG-3′. Restriction enzymes that recognize a site containing 5′-CG-3′ therefore cut human DNA relatively infrequently. Examples are *Sma*I (5′-CCCGGG-3′), which cuts human DNA on average once every 78 kb, and *Bss*HII (5′-GCGCGC-3′) which cuts once every 390 kb. Note that *Not*I, an eight-nucleotide cutter, also targets 5′-CG-3′ sequences (recognition sequence 5′-GCGGCCGC-3′) and cuts human DNA very rarely - approximately once every 10 Mb.

The sequence 5′-CG-3′ is rare in human DNA because of methylation of the C, followed by deamination to give T.

The potential of restriction mapping is therefore increased by using rare cutters. It is still not possible to construct restriction maps of the genomes of animals and plants, but it is feasible to use the technique with large cloned fragments,

and the smaller DNA molecules of prokaryotes and lower eukaryotes such as yeast and fungi.

If a rare cutter is used then it may be necessary to employ a special type of agarose gel electrophoresis to study the resulting restriction fragments. This is because the relationship between the length of a DNA molecule and its migration rate in an electrophoresis gel is not linear, the resolution decreasing as the molecules get longer. This means that it is not possible to separate molecules more than about 50 kb in length because all of these longer molecules run as a single slowly migrating band in a standard agarose gel. To separate them it is necessary to replace the linear electric field used in conventional gel electrophoresis with a more complex field. An example is provided by orthogonal field alternation gel electrophoresis (**OFAGE**), in which the electric field alternates between two pairs of electrodes, each positioned at an angle of 45° to the length of the gel. The DNA molecules still move down through the gel, but each change in the field forces the molecules to realign. Shorter molecules realign more quickly than longer ones and so migrate more rapidly through the gel. The overall result is that molecules much longer than those separated by conventional gel electrophoresis can be resolved. Related techniques include **CHEF** (contour clamped homogeneous electric fields) and **FIGE** (field inversion gel electrophoresis).

Conventional and non-conventional agarose gel electrophoresis. (A) In standard agarose gel electrophoresis the electrodes are placed at either end of the gel and the DNA molecules migrate directly towards the positive electrode. Molecules longer than (more...)

Direct examination of DNA molecules for restriction sites

It is also possible to use methods other than electrophoresis to map restriction sites in DNA molecules. With the technique called optical mapping (Schwartz *et al*., 1993), restriction sites are directly located by looking at the cut DNA molecules with a microscope (Figure 5.27). The DNA must first be attached to a glass slide in such a way that the individual molecules become stretched out, rather than clumped together in a mass. There are two ways of doing this: gel stretching and molecular combing. To prepare gel-stretched DNA fibers (Schwartz *et al*., 1993), chromosomal DNA is suspended in molten agarose and placed on a microscope slide. As the gel cools and solidifies, the DNA molecules become extended (Figure 5.28A). To utilize gel stretching in optical mapping, the microscope slide onto which the molten agarose is placed is first coated with a restriction enzyme. The enzyme is inactive at this stage because there are no magnesium ions, which the enzyme needs in order to function. Once the gel has solidified it is washed with a solution containing magnesium chloride, which activates the restriction enzyme. A fluorescent dye is added, such as DAPI (4,6-diamino-2-phenylindole dihydrochloride), which stains the

DNA so that the fibers can be seen when the slide is examined with a high-power fluorescence microscope. The restriction sites in the extended molecules gradually become gaps as the degree of fiber extension is reduced by the natural springiness of the DNA, enabling the relative positions of the cuts to be recorded.

Optical mapping. The image shows a 2.4-Mb segment of the *Deinococcus radiodurans* genome after treatment with the restriction endonuclease *Nhe*I. The positions of the cut sites are visible as gaps in the white strand of DNA. Reprinted with permission from (more...)

Gel stretching and molecular combing. (A) To carry out gel stretching, molten agarose containing chromosomal DNA molecules is pipetted onto a microscope slide coated with a restriction enzyme. As the gel solidifies, the DNA molecules become stretched. (more...)

In molecular combing (Michalet *et al*., 1997), the DNA fibers are prepared by dipping a silicone-coated cover slip into a solution of DNA, leaving it for 5 minutes (during which time the DNA molecules attach to the cover slip by their ends), and then removing the slip at a constant speed of 0.3 mm s^{-1}. The force required to pull the DNA molecules through the meniscus causes them to line up. Once in the air, the surface of the cover slip dries, retaining the DNA molecules as an array of parallel fibers.

Optical mapping was First applied to large DNA fragments cloned in YAC and BAC vectors (Section 4.2.1). More recently, the feasibility of using this technique with genomic DNA has been proven with studies of a 1-Mbchromosome of the malaria parasite *Plasmodium falciparum* (Jing *et al*., 1999), and the two chromosomes and single megaplasmid of the bacterium *Deinococcus radiodurans* (Lin *et al*., 1999).

5.3.2. Fluorescent *in situ* hybridization (FISH)

The optical mapping method described above provides a link to the second type of physical mapping procedure that we will consider - FISH (Heiskanen *et al*., 1996). As in optical mapping, FISH enables the position of a marker on a chromosome or extended DNA molecule to be directly visualized. In optical mapping the marker is a restriction site and it is visualized as a gap in an extended DNA fiber. In FISH, the marker is a DNA sequence that is visualized by hybridization with a fluorescent probe.

In situ *hybridization with radioactive or fluorescent probes*

In situ hybridization is a version of hybridization analysis in which an intact chromosome is examined by probing it with a labeled DNA molecule. The position on the chromosome at which hybridization occurs provides information

about the map location of the DNA sequence used as the probe. For the method to work, the DNA in the chromosome must be made single stranded ('denatured') by breaking the base pairs that

hold the double helix together. Only then will the chromosomal DNA be able to hybridize with the probe. The standard method for denaturing chromosomal DNA without destroying the morphology of the chromosome is to dry the preparation onto a glass microscope slide and then treat with formamide.

Fluorescent *in situ* hybridization. A sample of dividing cells is dried onto a microscope slide and treated with formamide so that the chromosomes become denatured but do not lose their characteristic metaphase morphologies (see Section 2.2.1). The position (more...)

In the early versions of *in situ* hybridization the probe was radioactively labeled but this procedure was unsatisfactory because it is difficult to achieve both sensitivity and resolution with a radioactive label, two critical requirements for successful *in situ* hybridization. Sensitivity requires that the radioactive label has a high emission energy (an example of such a radiolabel is ^{32}P), but if the radiolabel has a high emission energy then it scatters its signal and so gives poor resolution. High resolution is possible if a radiolabel with low emission energy, such as H, is used, but these have such low sensitivity that lengthy exposures are needed, leading to a high background and difficulties in discerning the genuine signal.

These problems were solved in the late 1980s by the development of non-radioactive fluorescent DNA labels. These labels combine high sensitivity with high resolution and are ideal for *in situ* hybridization. Fluorolabels with different colored emissions have been designed, making it possible to hybridize a number of different probes to a single chromosome and distinguish their individual hybridization signals, thus enabling the relative positions of the probe sequences to be mapped. To maximize sensitivity, the probes must be labeled as heavily as possible, which in the past has meant that they must be quite lengthy DNA molecules - usually cloned DNA fragments of at least 40 kb. This requirement is less important now that techniques for achieving heavy labeling with shorter molecules have been developed. As far as the construction of a physical map is concerned, a cloned DNA fragment can be looked upon as simply another type of marker, although in practice the use of clones as markers adds a second dimension because the cloned DNA is the material from which the DNA sequence is determined. Mapping the positions of clones therefore provides a direct link between a genome map and its DNA sequence.

If the probe is a long fragment of DNA then one potential problem, at least with higher eukaryotes, is that it is likely to contain examples of repetitive DNA sequences (Section 2.4) and so may hybridize to many chromosomal positions, not just the specific point to which it is perfectly matched. To reduce this non-

specific hybridization, the probe, before use, is mixed with unlabeled DNA from the organism being studied. This DNA can simply be total nuclear DNA (i.e. representing the entire genome) but it is better if a fraction enriched for repeat sequences is used. The idea is that the unlabeled DNA hybridizes to the repetitive DNA sequences in the probe, blocking these so that the subsequent *in situ* hybridization is driven wholly by the unique sequences (Lichter *et al*., 1990). Non-specific hybridization is therefore reduced or eliminated entirely.

A method for blocking repetitive DNA sequences in a hybridization probe. In this example the probe molecule contains two genome-wide repeat sequences (shown in green). If these sequences are not blocked then the probe will hybridize non-specifically
to (more...)

FISH in action

FISH was originally used with metaphase chromosomes. These chromosomes, prepared from nuclei that are undergoing division, are highly condensed and each chromosome in a set takes up a recognizable appearance, characterized by the position of its centromere and the banding pattern that emerges after the chromosome preparation is stained. With metaphase chromosomes, a fluorescent signal obtained by FISH is mapped by measuring its position relative to the end of the short arm of the chromosome (the FLpter value). A disadvantage is that the highly condensed nature of metaphase chromosomes means that only low-resolution mapping is possible, two markers having to be at least 1 Mb apart to be resolved as separate hybridization signals (Trask *et al*., 1991). This degree of resolution is insufficient for the construction of useful chromosome maps, and the main application of metaphase FISH has been in determining the chromosome on which a new marker is located, and providing a rough idea of its map position, as a preliminary to finer scale mapping by other methods.

For several years these 'other methods' did not involve any form of FISH, but since 1995 a range of higher resolution FISH techniques has been developed. With these techniques, higher resolution is achieved by changing the nature of the chromosomal preparation being studied. If metaphase chromosomes are too condensed for fine -scale mapping then we must use chromosomes that are more extended. There are two ways of doing this (Heiskanen *et al*., 1996):

Mechanically stretched chromosomes can be obtained by modifying the preparative method used to isolate chromosomes from metaphase nuclei. The inclusion of a centrifugation step generates shear forces which can result in the chromosomes becoming stretched to up to 20 times their normal length. Individual chromosomes are still recognizable and FISH signals can be mapped in the same way as with normal metaphase

chromosomes. The resolution is significantly improved and markers that are 200–300 kb apart can be distinguished.

Non-metaphase chromosomes can be used because it is only during metaphase that chromosomes are highly condensed: at other stages of the cell cycle the chromosomes are naturally unpacked. Attempts have been made to use prophase nuclei because in these the chromosomes are still sufficiently condensed for individual ones to be identified. In practice, however, these preparations provide no advantage over mechanically stretched chromosomes. Interphase chromosomes are more useful because this stage of the cell cycle (between nuclear divisions) is when the chromosomes are most unpacked. Resolution down to 25 kb is possible, but chromosome morphology is lost so there are no external reference points against which to map the position of the probe. This technique is therefore used after preliminary map information has been obtained, usually as a means of determining the order of a series of markers in a small region of a chromosome.

Interphase chromosomes contain the most unpacked of all cellular DNA molecules. To improve the resolution of FISH to better than 25 kb it is therefore necessary to abandon intact chromosomes and instead use purified DNA. This approach, called fiber-FISH, makes use of DNA prepared by gel stretching or molecular combing and can distinguish markers that are less than 10 kb apart.

5.3.3. Sequence tagged site (STS) mapping

To generate a detailed physical map of a large genome we need, ideally, a high-resolution mapping procedure that is rapid and not technically demanding. Neither of the two techniques that we have considered so far - restriction mapping and FISH - meets these requirements. Restriction mapping is rapid, easy, and provides detailed information, but it cannot be applied to large genomes. FISH can be applied to large genomes, and modified versions such as fiber-FISH can give high-resolution data, but FISH is difficult to carry out and data accumulation is slow, map positions for no more than three or four markers being obtained in a single experiment. If detailed physical maps are to become a reality then we need a more powerful technique.

At present the most powerful physical mapping technique, and the one that has been responsible for generation of the most detailed maps of large genomes, is STS mapping. A sequence tagged site or **STS** is simply a short DNAsequence, generally between 100 and 500 bp in length, that is easily recognizable and occurs only once in the chromosome or genome being studied. To map a set of STSs, a collection of overlapping DNA fragments from a single chromosome or from the entire genome is needed. In the example shown in a fragment collection has been prepared from a single chromosome, with each point along the chromosome represented on average five times in the collection. The data from which the map will be derived are obtained by determining which

fragments contain which STSs. This can be done by hybridization analysis but PCR is generally used because it is quicker and has proven to be more amenable to automation. The chances of two STSs being present on the same fragment will, of course, depend on how close together they are in the genome. If they are very close then there is a good chance that they will always be on the same fragment; if they are further apart then sometimes they will be on the same fragment and sometimes they will not. The data can therefore be used to calculate the distance between two markers, in a manner analogous to the way in which map distances are determined by linkage analysis. Remember that in linkage analysis a map distance is calculated from the frequency at which crossovers occur between two markers. STS mapping is essentially the same, except that each map distance is based on the frequency at which *breaks* occur between two markers.

A fragment collection suitable for STS mapping. The fragments span the entire length of a chromosome, with each point on the chromosome present in an average of five fragments. The two blue markers are close together on the chromosome map and there is (more...)

The description of STS mapping given above leaves out some critical questions: What exactly is an STS? How is the DNA fragment collection obtained?

Any unique DNA sequence can be used as an STS

To qualify as an STS, a DNA sequence must satisfy two criteria. The first is that its sequence must be known, so that a PCR assay can be set up to test for the presence or absence of the STS on different DNA fragments. The second requirement is that the STS must have a unique location in the chromosome being studied, or in the genome as a whole if the DNA fragment set covers the entire genome. If the STS sequence occurs at more than one position then the mapping data will be ambiguous. Care must therefore be taken to ensure that STSs do not include sequences found in repetitive DNA.

These are easy criteria to satisfy and STSs can be obtained in many ways, the most common sources being **expressed sequence tags (ESTs)**, SSLPs, and **random genomic sequences**.

Expressed sequence tags ***(ESTs)***. These are short sequences obtained by analysis of cDNA clones (Marra *et al*., 1998). Complementary DNA is prepared by converting an mRNA preparation into double-stranded DNA. Because the mRNA in a cell is derived from protein-coding genes, cDNAs and the ESTs obtained from them represent the genes that were being expressed in the cell from which the mRNA was prepared. ESTs are looked upon as a rapid means of gaining access to the sequences of important genes, and they are valuable even if their sequences are incomplete. An EST can also be used as an STS, assuming that it

comes from a unique gene and not from a member of a gene family in which all the genes have the same or very similar sequences.

SSLPs . we examined the use of microsatellites and other SSLPs in genetic mapping. SSLPs can also be used as STSs in physical mapping. SSLPs that are polymorphic and have already been mapped by linkage analysis are particularly valuable as they provide a direct connection between the genetic and physical maps.

Random genomic sequences . These are obtained by sequencing random pieces of cloned genomic DNA, or simply by downloading sequences that have been deposited in the databases.

One method for preparing cDNA. Most eukaryotic mRNAs have a poly(A) tail at their 3′ end (Section 10.1.2). This series of A nucleotides is used as the priming site for the first stage of cDNA synthesis, carried out by reverse transcriptase - a (more...)

Fragments of DNA for STS mapping

The second component of an STS mapping procedure is the collection of DNA fragments spanning the chromosome or genome being studied. This collection is sometimes called the mapping reagent and at present there are two ways in which it can be assembled: as a clone library and as a panel of radiation hybrids. We will consider radiation hybrids first.

A radiation hybrid is a rodent cell that contains fragments of chromosomes from a second organism (McCarthy, 1996). The technology was first developed in the 1970s when it was discovered that exposure of human cells to X-ray doses of 3000–8000 rads causes the chromosomes to break up randomly into fragments, larger X-ray doses producing smaller fragments. This treatment is of course lethal for the human cells, but the chromosome fragments can be propagated if the irradiated cells are subsequently fused with non-irradiated hamster or other rodent cells. Fusion is stimulated either chemically with polyethylene glycol or by exposure to Sendai virus. Not all of the hamster cells take up chromosome fragments so a means of identifying the hybrids is needed. The routine selection process is to use a hamster cell line that is unable to make either thymidine kinase (TK) or hypoxanthine phosphoribosyl transferase (HPRT), deficiencies in either of these two enzymes being lethal when the cells are grown in a medium containing a mixture of hypoxanthine, aminopterin and thymidine (HAT medium). After fusion, the cells are placed in HAT medium. Those that grow are hybrid hamster cells that have acquired human DNA fragments that include genes for the human TK and HPRT enzymes, which are synthesized inside the hybrids, enabling these cells to grow in the selective medium. The treatment

results in hybrid cells that contain a random selection of human DNA fragments inserted into the hamster chromosomes. Typically the fragments are 5–10 Mb in size, with each cell containing fragments equivalent to 15–35% of the human genome. The collection of cells is called a radiation hybrid panel and can be used as a mapping reagent in STS mapping, provided that the PCR assay used to identify the STS does not amplify the equivalent region of DNA from the hamster genome.

Radiation hybrids. (A) The result of irradiation of human cells: the chromosomes break into fragments, smaller fragments generated by higher X-ray doses. In (B), a radiation hybrid is produced by fusing an irradiated human cell with an untreated hamster (more...)

The radiation hybrid map of the mouse genome. Physical mapping is a prelude to sequencing of the mouse genome and enables comparisons to be made between mouse and human chromosomes. Completion of the human genome sequence is not the only objective of (more...)

A second type of radiation hybrid panel, containing DNA from just one human chromosome, can be constructed if the cell line that is irradiated is not a human one but a second type of rodent hybrid. Cytogeneticists have developed a number of rodent cell lines in which a single human chromosome is stably propagated in the rodent nucleus. If a cell line of this type is irradiated and fused with hamster cells, then the hybrid hamster cells obtained after selection will contain either human or mouse chromosome fragments, or a mixture of both. The ones containing human DNA can be identified by probing with a human-specific genome-wide repeat sequence, such as the short interspersed nuclear element (SINE) called Alu (Section 2.4.2), which has a copy number of just over 1 million (see Table 1.2) and so occurs on average once every 4 kb in the human genome. Only cells containing human DNA will hybridize to Alu probes, enabling the uninteresting mouse hybrids to be discarded and STS mapping to be directed at the cells containing human chromosome fragments.

Radiation hybrid mapping of the human genome was initially carried out with chromosome-specific rather than whole-genome panels because it was thought that fewer hybrids would be needed to map a single chromosome than would be needed to map the entire genome. It turns out that a high-resolution map of a single human chromosome requires a panel of 100–200 hybrids, which is about the most that can be handled conveniently in a PCR screening program. But whole-genome and single-chromosome panels are constructed differently, the former involving irradiation of just human DNA, and the latter requiring irradiation of a mouse cell containing much mouse DNA and relatively little human DNA. This means that the human DNA content per hybrid is much lower in a single-chromosome panel than in a whole-genome panel. It transpires that detailed mapping of the entire human genome is possible with fewer than 100 whole-genome radiation hybrids, so whole-genome mapping is no more difficult than single-chromosome mapping. Once this was realized, whole-genome

radiation hybrids became a central component of the mapping phase of the Human Genome Project (Section 6.3.1).

Whole-genome libraries are also being used for STS mapping of other mammalian genomes and for those of the zebra fish and the chicken (McCarthy, 1996).

A clone library can also be used as the mapping reagent for STS analysis

A preliminary to the sequencing phase of a genome project is to break the genome or isolated chromosomes into fragments and to clone each one in a high-capacity vector, one able to handle large fragments of DNA. This results in a clone library, a collection of DNA fragments, which, in this case, have an average size of several hundred kb. As well as supporting the sequencing work, this type of clone library can also be used as a mapping reagent in STS analysis.

As with radiation hybrid panels, a clone library can be prepared from genomic DNA, and so represents the entire genome, or a chromosome-specific library can be made if the starting DNA comes from just one type of chromosome. The latter is possible because individual chromosomes can be separated by flow cytometry. To carry out this technique, dividing cells (ones with condensed chromosomes) are carefully broken open so that a mixture of intact chromosomes is obtained. The chromosomes are then stained with a fluorescent dye. The amount of dye that a chromosome binds depends on its size, so larger chromosomes bind more dye and fluoresce more brightly than smaller ones. The chromosome preparation is diluted and passed through a fine aperture, producing a stream of droplets, each one containing a single chromosome. The droplets pass through a detector that measures the amount of fluorescence, and hence identifies which droplets contain the particular chromosome being sought. An electric charge is applied to these drops, and no others, enabling the droplets containing the desired chromosome to be deflected and separated from the rest. What if two different chromosomes have similar sizes, as is the case with human chromosomes 21 and 22? These can usually be separated if the dye that is used is not one that binds non-specifically to DNA, but instead has a preference for AT- or GC-rich regions. Examples of such dyes are Hoechst 33258 and chromomycin A_3, respectively. Two chromosomes that are the same size rarely have identical GC contents, and so can be distinguished by the amounts of AT- or GC-specific dye that they bind.

Separating chromosomes by flow cytometry. A mixture of fluorescently stained chromosomes is passed through a small aperture so that each drop that emerges contains just one chromosome. The fluorescence detector identifies the signal from drops containing (more...)

Compared with radiation hybrid panels, clone libraries have one important advantage for STS mapping. This is the fact that the individual clones can

subsequently provide the DNA that is actually sequenced. The data resulting from STS analysis, from which the physical map is generated, can equally well be used to determine which clones contain overlapping DNA fragments, enabling a clone contig to be built up; for other methods for assembling clone contigs see Section 6.2.2). This assembly of overlapping clones can be used as the base material for a lengthy, continuous DNA sequence, and the STS data can later be used to anchor this sequence precisely onto the physical map. If the STSs also include SSLPs that have been mapped by genetic linkage analysis then the DNA sequence, physical map and genetic map can all be integrated.

The value of clone libraries in genome projects. The small clone library shown in this example contains sufficient information for an STS map to be constructed, and can also be used as the source of the DNA that will be sequenced.

Extraction, Isolation, Seperation, Purification and Identification of bioactive compounds

Introduction

According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation.

Extraction

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses (Fabricant and Farnsworth, 2001), then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll (Cos et al., 2006).

As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods,

such as sonification, heating under reflux, soxhlet extraction and others are commonly used (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001) for the plant samples extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems. A brief summary of the experimental conditions for the various methods.

Fourier-transform infrared spectroscopy (FTIR*)*

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt et al., 2007; Hazra et al., 2007). In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be

identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) to and then compressed into a thin pellet which can be analyzed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the solution then placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate.

Cryopreservation

Cryo-preservation or **cryo-conservation** is a process where organelles, cells, tissues, extracellular matrix, organs or anyother biological constructs susceptible to damage caused by unregulated chemical kinetics are preserved by cooling to very low temperatures (typically −80 °C using solid carbon dioxide or −196 °C using liquid nitrogen). At low enough temperatures, any enzymatic or chemical activity which might cause damage to the biological material in question is effectively stopped. Cryopreservation methods seek to reach low temperatures without causing additional damage caused by the formation of ice crystals during freezing. Traditional cryopreservation has relied on coating the material to be frozen with a class of molecules termed cryoprotectants. New methods are constantly being investigated due to the inherent toxicity of many cryoprotectants. By default it should be considered that cryopreservation alters or compromises the structure and function of cells unless it is proven otherwise for a particular cell population. Cryoconservation of animal genetic resources is the process in which animal genetic material is collected and stored with the intention of conservation of the breed.

Natural cryopreservation

Water-bears (*Tardigrada*), microscopic multicellular organisms, can survive freezing by replacing most of their internal water with the sugar trehalose, preventing it from crystallization that otherwise damages cell membranes. Mixtures of solutes can achieve similar effects. Some solutes, including salts, have the disadvantage that they may be toxic at intense concentrations. In addition to the water-bear, wood frogs can tolerate the freezing of their blood and other tissues. Urea is accumulated in tissues in preparation for overwintering, and liver glycogen is converted in large quantities to glucose in response to internal ice formation. Both urea and glucose act as "cryoprotectants" to limit the amount of ice that forms and to reduce osmotic shrinkage of cells. Frogs can survive many freeze/thaw events during winter if no more than about 65% of the total body water
freezes. Research exploring the phenomenon of "freezing frogs" has been performed primarily by the Canadian researcher, Dr. Kenneth B. Storey.
Freeze tolerance, in which organisms survive the winter by freezing solid and ceasing life functions, is known in a few vertebrates: five species of frogs (*Rana sylvatica*, *Pseudacris triseriata*, *Hyla crucifer*, *Hyla versicolor*, *Hyla chrysoscelis*), one of salamanders (*Hynobius keyserlingi*), one of snakes (*Thamnophis sirtalis*) and three of turtles (*Chrysemys picta*, *Terrapene carolina*, *Terrapene ornata*). Snapping turtles *Chelydra serpentina* and wall lizards *Podarcis muralis* also survive nominal freezing but it has not been established to be adaptive for overwintering. In the case of *Rana sylvatica* one cryopreservant is ordinary glucose, which increases in concentration by approximately 19 mmol/l when the frogs are cooled slowly.

Temperature

Storage at very cold temperatures is presumed to provide an indefinite longevity to cells, although the actual effective life is rather difficult to prove. Researchers experimenting with dried seeds found that there was noticeable variability of deterioration when samples were kept at different temperatures – even ultra-cold temperatures. Temperatures less than the glass transition point (Tg) of polyol's water solutions, around −136 °C (137 K; −213 °F), seem to be accepted as the range where biological activity very substantially slows, and −196 °C (77 K; −321 °F), the boiling point of liquid nitrogen, is the preferred temperature for storing important specimens. While refrigerators, freezers and extra-cold

freezers are used for many items, generally the ultra-cold of liquid nitrogen is required for successful preservation of the more complex biological structures to virtually stop all biological activity.

Risks

Phenomena which can cause damage to cells during cryopreservation mainly occur during the freezing stage, and include: solution effects, extracellular ice formation, dehydration and intracellular ice formation. Many of these effects can be reduced by cryoprotectants. Once the preserved material has become frozen, it is relatively safe from further damage. However, estimates based on the accumulation of radiation-induced
DNA damage during cryonic storage have suggested a maximum storage period of 1000 years.

Solution effects

As ice crystals grow in freezing water, solutes are excluded, causing them to become concentrated in the remaining liquid water. High concentrations of some solutes can be very damaging.

Extracellular ice formation

When tissues are cooled slowly, water migrates out of cells and ice forms in the extracellular space. Too much extracellular ice can cause mechanical damage to the cell membrane due to crushing.

Dehydration

Migration of water, causing extracellular ice formation, can also cause cellular dehydration. The associated stresses on the cell can cause damage directly.

Intracellular ice formation

While some organisms and tissues can tolerate some extracellular ice, any appreciable intracellular ice is almost always fatal to cells.

Main methods to prevent risks

The main techniques to prevent cryopreservation damages are a well established combination of *controlled rate and slow freezing* and a newer flash-freezing process known as *vitrification*.

Slow programmable freezing

A tank of liquid nitrogen, used to supply a cryogenic freezer (for storing laboratory samples at a temperature of about −150 °C)

Controlled-rate and slow freezing, also known as *slow programmable freezing (SPF)*, is a set of well established techniques developed during the early 1970s which enabled the first human embryo frozen birth Zoe Leyland during 1984. Since then, machines that freeze biological samples using programmable sequences, or controlled rates, have been used all over the world for human, animal and cell biology – "freezing down" a sample to better preserve it for eventual thawing, before it is frozen, or cryopreserved, in liquid nitrogen. Such machines are used for freezing oocytes, skin, blood products, embryo, sperm, stem cells and general tissue preservation in hospitals, veterinary practices and research laboratories around the world. As an example, the number of live births from frozen embryos 'slow frozen' is estimated at some 300,000 to 400,000 or 20% of the estimated 3 million in vitro fertilisation (IVF) births.

Lethal intracellular freezing can be avoided if cooling is slow enough to permit sufficient water to leave the cell during progressive freezing of the extracellular fluid. To minimize the growth of extracellular ice crystal growth and recrystallization, biomaterials such as alginates, polyvinyl alcohol or chitosan can be used to impede ice crystal growth along with traditional small molecule cryoprotectants. That rate differs between cells of differing size and water permeability: a typical cooling rate of about 1 °C/minute is appropriate for many mammalian cells after treatment with cryoprotectants such as glycerol or dimethyl sulfoxide, but the rate is not a universal optimum. The 1 °C / minute rate can be achieved by using devices such as a rate-controlled freezer or a benchtop portable freezing container.

Several independent studies have provided evidence that frozen embryos stored using slow-freezing techniques may in some ways be 'better' than fresh in IVF. The studies indicate that using frozen embryos and eggs rather than fresh embryos and eggs reduced the risk of stillbirth and premature delivery though the exact reasons are still being explored.

Vitrification

Researchers Greg Fahy and William F. Rall helped to introduce vitrification to reproductive cryopreservation in the mid-1980s.As of 2000, researchers claim vitrification provides the benefits of cryopreservation without damage due to ice

crystal formation. The situation became more complex with the development of tissue engineering as both cells and biomaterials need to remain ice-free to preserve high cell viability and functions, integrity of constructs and structure of biomaterials. Vitrification of tissue engineered constructs was first reported by Lilia Kuleshova, who also was the first scientist to achieve vitrification of woman's eggs (oocytes), which resulted in live birth in 1999. For clinical cryopreservation, vitrification usually requires the addition of cryoprotectants prior to cooling. The cryoprotectants act like antifreeze: they decrease the freezing temperature. They also increase the viscosity. Instead of crystallizing, the syrupy solution becomes an amorphous ice—it *vitrifies*. Rather than a phase change from liquid to solid by crystallization, the amorphous state is like a "solid liquid", and the transformation is over a small temperature range described as the "glass transition" temperature.

Vitrification of water is promoted by rapid cooling, and can be achieved without cryoprotectants by an extremely rapid decrease of temperature (megakelvins per second). The rate that is required to attain glassy state in pure water was considered to be impossible until 2005.Two conditions usually required to allow vitrification are an increase of the viscosity and a decrease of the freezing temperature. Many solutes do both, but larger molecules generally have a larger effect, particularly on viscosity. Rapid cooling also promotes vitrification.

For established methods of cryopreservation, the solute must penetrate the cell membrane in order to achieve increased viscosity and decrease freezing temperature inside the cell. Sugars do not readily permeate through the membrane. Those solutes that do, such as dimethyl sulfoxide, a common cryoprotectant, are often toxic in intense concentration. One of the difficult compromises of vitrifying cryopreservation concerns limiting the damage produced by the cryoprotectant itself due to cryoprotectant toxicity. Mixtures of cryoprotectants and the use of ice blockers have enabled the Twenty-First Century Medicine company to vitrify a rabbit kidney to −135 °C with their proprietary vitrification mixture. Upon rewarming, the kidney was transplanted successfully into a rabbit, with complete functionality and viability, able to sustain the rabbit indefinitely as the sole functioning kidney.

Preservation of microbiology cultures

Bacteria and fungi can be kept short-term (months to about a year, depending) refrigerated, however, cell division and metabolism is not completely arrested and thus is not an optimal option for long-term storage (years) or to preserve cultures genetically or phenotypically, as cell divisions can lead to mutations or

sub-culturing can cause phenotypic changes. A preferred option, species-dependent, is cryopreservation. Nematode worms are the only multicellular eukaryotes that have been shown to survive cryopreservation.

Fungi

Fungi, notably zygomycetes, ascomycetes and higher basidiomycetes, regardless of sporulation, are able to be stored in liquid nitrogen or deep-frozen. Crypreservation is a hallmark method for fungi that do not sporulate (otherwise other preservation methods for spores can be used at lower costs and ease), sporulate but have delicate spores (large or freeze-dry sensitive), are pathogenic (dangerous to keep metabolically active fungus) or are to be used for genetic stocks (ideally to have identical composition as the original deposit). As with many other organisms, cryoprotectants like DMSO or glycerol (e.g. filamentous fungi 10% glycerol or yeast 20% glycerol) are used. Differences between choosing cryoprotectants are species (or class) dependent, but generally for fungi penetrating cryoprotectants like DMSO, glycerol or polyethylene glycol are most effective (other non-penetrating ones include sugars mannitol, sorbitol, dextran, etc.). Freeze-thaw repetition is not recommended as it can decrease viability. Back-up deep-freezers or liquid nitrogen storage sites are recommended. Multiple protocols for freezing are summarized below (each uses screw-cap polypropylene cryotubes):

Bacteria

Many common culturable laboratory strains are deep-frozen to preserve genetically and phenotypically stable, long-term stocks. Sub-culturing and prolonged refrigerated samples may lead to loss of plasmid(s) or mutations. Common final glycerol percentages are 15, 20 and 25. From a fresh culture plate, one single colony of interest is chosen and liquid culture is made. From the liquid culture, the medium is directly mixed with equal amount of glycerol; the colony should be checked for any defects like mutations. All antibiotics should be washed from the culture before long-term storage. Methods vary, but mixing can be done gently by inversion or rapidly by vortex and cooling can vary by either placing the cryotube directly at −50 to −95 °C, shock-freezing in liquid nitrogen or gradually cooling and then storing at −80 °C or cooler (liquid nitrogen or liquid nitrogen

vapor). Recovery of bacteria can also vary, namely if beads are stored within the tube then the few beads can be used to plate or the frozen stock can be scraped with a loop and then plated, however, since only little stock is needed the entire tube should never be
completely thawed and repeated freeze-thaw should be avoided. 100% recovery is not feasible regardless of methodology.

Worms

The microscopic soil-dwelling nematode roundworms Panagrolaimus detritophagus and Plectus parvus are the only eukaryotic organisms that have been proven to be viable after long-term cryopreservation to date. In this case, the preservation was natural rather than artificial, due to permafrost.

Freezable tissues

Generally, cryopreservation is easier for thin samples and small clumps of individual cells, because these can be cooled more quickly and so require lesser doses of toxic cryoprotectants. Therefore, cryopreservation of human livers and hearts for storage and transplant is still impractical.

Nevertheless, suitable combinations of cryoprotectants and regimes of cooling and rinsing during warming often allow the successful cryopreservation of biological materials, particularly cell suspensions or thin tissue samples. Examples include:

Semen in semen cryopreservation
Blood
Special cells for transfusion
Stem cells. It is optimal in high concentration of synthetic serum, stepwise equilibration and slow cooling.

Umbilical cord blood *Further information: Cord blood bank#Cryopreservation*
Tissue samples like tumors and histological cross sections
Eggs (oocytes) in oocyte cryopreservation
Embryos at cleavage stage (that are 2, 4 or 8 cells) or at blastocyst stage, in embryo cryopreservation
Ovarian tissue in ovarian tissue cryopreservation
Plant seeds or shoots may be cryopreserved for conservation purposes

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CLASS: IMSc MB COURSE NAME: MARINE MICROBIOLOGY

COURSE CODE: 18MBP105A UNIT: V BATCH-2018-2020

Sl. No	QUESTIONS	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWERS
1	Half life (t ½) is the time required to:	Change the amount of a drug in plasma by half during elimination	Metabolize a half of an introduced drug into the active metabolite	Absorb a half of an introduced drug	Bind a half of an introduced drug to plasma proteins	Change the amount of a drug in plasma by half during elimination
2	Aspirin is chemically	Sodium salicylate	Acetylsalicylic acid	Salicylamide	Sodium salicylamide	Acetylsalicylic acid
3	Which is the most appropriate to the term “receptor”	All types of ion channels modulated by a drug	Enzymes of oxidizing-reducing reactions activated by a drug	Active macromolecular components of a cell or an organism which a drug molecule has to combine with in order to elicit its specific effect	Carriers activated by a drug	Active macromolecular components of a cell or an organism which a drug molecule has to combine with in order to elicit its specific effect
4	What does “affinity” mean?	A measure of how tightly a drug binds to plasma proteins	A measure of how tightly a drug binds to a receptor	A measure of inhibiting potency of a drug	A measure of bioavailability of a drug	A measure of how tightly a drug binds to a receptor
5	A measure of bioavailability of a	A measure of how	An agonist is a	Interacts with	Interacts with	Increases

	drug	tightly a drug binds to a receptor	substance that:	the receptor without producing any effect	the receptor and initiates changes in cell function, producing various effects	concentration of another substance to produce effect
6	An agonist is a substance that:	Interacts with the receptor without producing any effect	Interacts with the receptor and initiates changes in cell function, producing various effects	Increases concentration of another substance to produce effect	Interacts with plasma proteins and doesn't produce any effect	Interacts with the receptor and initiates changes in cell function, producing various effects
7	An antagonist is a substance that:	Binds to the receptors and initiates changes in cell function, producing maximal effect	Binds to the receptors and initiates changes in cell function, producing submaximal effect	Interacts with plasma proteins and doesn't produce any effect	Binds to the receptors without directly altering their functions	Binds to the receptors without directly altering their functions
8	A competitive antagonist is a substance that:	Interacts with receptors and produces sub maximal effect	Binds to the same receptor site and progressively inhibits the agonist response	Binds to the nonspecific sites of tissue	Binds to one receptor subtype as an agonist and to another as an antagonist	Binds to the same receptor site and progressively inhibits the agonist response

9	The substance binding to one receptor subtype as an agonist and to another as an antagonist is called:	Competitive antagonist	Irreversible antagonist	Agonist-antagonist	Partial agonist	Agonist-antagonist
10	Irreversible interaction of an antagonist with a receptor is due to:	Ionic bonds	Hydrogen bonds	Covalent bonds	Weak bonds	Covalent bonds
11	Tick the second messenger of G-protein-coupled (metabotropic) receptor:	Adenylyl cyclase	Sodium ions	Phospholipase C	cAMP	cAMP
12	What is the type of drug-to-drug interaction which is connected with processes of absorption, biotransformation, distribution and excretion?	Pharmacodynamic interaction	Physical and chemical interaction	Pharmaceutical interaction	Pharmacokinetic interaction	Pharmacokinetic interaction
13	Chloramphenicol is derived from	Streptomyces venezulae	Streptomyces griseus	Streptomyces kanamycin	Pencillin	Streptomyces griseu
14	A hydrophilic medicinal agent has the following property:	Low ability to penetrate through the cell membrane lipids	Penetrate through membranes by means of endocytosis	Easy permeation through the blood-brain barrier	High reabsorption in renal tubules	Low ability to penetrate through the cell membrane lipids
15	The feature of the sublingual route:	Pretty fast absorption	A drug is exposed to gastric secretion	A drug is exposed more prominent liver	A drug can be administrated in a variety of	Pretty fast absorptio

				metabolism	doses	
16	Pick out the parenteral route of medicinal agent administration:	Rectal	Oral	Sublingual	Inhalation	Inhalation
17	Parenteral administration:	Cannot be used with unconsciousness patients	Generally results in a less accurate dosage than oral administration	Usually produces a more rapid response than oral administration	Is too slow for emergency use	Usually produces a more rapid response than oral administration
18	Volume of distribution (Vd) one must take into _____	Concentration of a substance in plasma	Concentration of substance in urine	Therapeutical width of drug action	A daily dose of drug	Concentration of a substance in plasma
19	Biotransformation of the drugs is to render them:	Less ionized	More pharmacologically active	More lipid soluble	Less lipid soluble	Less lipid soluble
20	Tick the drug type for which microsomal oxidation is the most prominent:	Lipid soluble	Water soluble	Low molecular weight	High molecular weight	Lipid soluble
21	Cell surface receptors are	C protein coupled receptors	G-protein coupled receptors	Protein A tyrosine kinases	Protein A B tyrosine kinase	G-protein coupled receptors
22	The receptor serves as	Recognition molecule	Non recognition molecule	Target sites	Active sites	Recognition molecule
23	Which one of the following not bound to membrane?	Tyrosine linked receptors	Steroid receptors	ion channel linked	G- protein coupled	steroid receptors

				receptors	receptors	
24	When the person remains well only when he is taking the drug is termed as the State of	psychic dependence	physical dependence	withdrawal syndrome	Non Psychic dependence	physical dependence
25	If the abusing drug is withdrawn the person develops	Abstinence	physical dependence	Tolerance	psychic dependence	Abstinence
26	If a greaster dose of the drug is required to elicit the normal pharmachological Effect the state is known as	dependence	abstinence	tolerance	intolerance	tolerance
27	Th 1 cells	enhance CMI	enhance humoral immunity	inhibit CMI	inhibit humoral immunity	enhance CMI
28	If the drug is disposed more speedily the state is known as	pharmacokinetic tolerance	pharmacodynamic tolerance	psychic tolerance	drug intolerance	pharmacokinetic tolerance
29	A repeated injection of egg albumin in such an animalcauses a violent reaction Called	cytotoxic type reaction	cell mediated reaction	immune complex mediated reaction	anaphylaxis	Anaphylaxis
30	A state where some changes develop in the tissue leading to less pharmacological Effect of the drug is known as	pharmacokinetic tolerance	pharmacodynamic tolerance	psychic tolerance	drug intolerance	pharmacodynamic tolerance
31	Best example of psychic dependence is	cigarette smoking	barbiturates	sulphonamides	salicylates	cigarette smoking

32	The state when the person seeks drugs purely for psychological pleasure is	drug dependence	physical dependence	psychic dependence	pathological equilibrium	psychic dependence
33	Substances like lead can remain deposited in bones without producing toxic effects Which is called	passive immunization	additive effect	antagonism	synergism	passive immunization
34	Inflammatory reactions initiated by mononuclear lymphocytes and not by Antibody alone are called	type I hypersensitivity	type II hypersensitivity	delayed hypersensitivity	type III hypersensitivity	delayed hypersensitivity
35	Methadone is	agonist of opioid receptors	antagonist of opioid receptors	agonist of μ receptors	antagonist of μ receptors	agonist of opioid receptors
36	Opioids used for abusing are by themselves	CNS stimulants	CNS depressants	CVS stimulants	CVS depressants	CNS depressants
37	The drug naltrexone is	agonist of opioid receptors	antagonist of opioid receptors	agonist of μ receptors	antagonist of μ receptors	antagonist of opioid receptors
38	The drugs used to treat abusing of opioids is	Ibu brufen	methadone	Diclofenac	Analgesic	Methadone
39	If the opioid abusers are doctors,nurses and other health workers The choice of drug used for treatment is	methadone	methadyl acetate	naltrexone	pethidine	Pethidine
40	Amphetamine is an	antifatigue agent	fatigue agent	nausea inducer	heroin	antifatigue agent
41	Polydrug abuse common in USA	cocaine and heroin	heroin and	amphetamine	nicotine	cocaine and heroin

	is		amphetamine	and cocaine		
42	The half life of cocaine is	2 hrs	3 hrs	15 hrs	1hr	1hr
43	Drug used for the treatment of acute cocaine overdose is	Naproxen	amphetamine	diazepam	Ibu brufen	diazepam
44	The mechanism of action of labetalol used for acute cocaine overdose is	blocking of Ca2+ channel	blocking of α and β receptor	blocking of K+ channel	blocking of P+ channel	blocking of α and β receptor
45	The drug of choice for CNS complications due to acute cocaine overdose is	labetalol	nifedipine	diazepam	sulphonamides	diazepam
46	The craving of cocaine is reduced by	labetalol	nifedipine	desipramine	diazepam	Desipramine
47	Cryopreservation is _____	Heating in liquid nitrogen	Freezing in liquid nitrogen	Drying in liquid nitrogen	Steaming in liquid nitrogen	Freezing in liquid nitrogen
48	In Cryopreservation, the microorganisms of culture are rapidly frozen in liquid nitrogen at_____	-72°C	-86°C	-196°C	-96°C	-196°C
49	The stabilizing agents used in cryopreservation is _____	Glycerol	Phenol	Terpenol	Lysol	Glycerol
50	_____that prevent the formation of ice crystals and promote cell survival.	Glycerol	Phenol	Terpenol	Lysol	Glycerol
51	Lyophilization is	Freeze etching	Freeze drying	Freeze	Freeze liquid	Freeze drying

	otherwiseknown as _____(Freeze-Drying)			shadowing	nitrogen	
52	In Lyophilization, the culture is rapidly frozen at _____	-196°C	-86°C	-70°C	-96°C	-70°C
53	The metabolic activities of microbial cells are stopped in lyophilization method by_____	dry dehydration	vacuum dehydration	Spray dehydration	Dry heat dehydration	vacuum dehydration
54	Lyophilized or freeze-dried pure cultures sealed and stored in the dark at_____	1°C	8°C	7°C	4°C	4°C
55	Half life (t ½) is the time required to:	Change the amount of a drug in plasma by half during elimination	Metabolize a half of an introduced drug into the active metabolite	Absorb a half of an introduced drug	Bind a half of an introduced drug to plasma proteins	Change the amount of a drug in plasma by half during elimination
56	Aspirin is chemically	Sodium salicylate	Acetylsalicylic acid	Salicylamide	Sodium salicylamide	Acetylsalicylic acid
57	Which is the most appropriate to the term “receptor”	All types of ion channels modulated by a drug	Enzymes of oxidizing-reducing reactions activated by a drug	Active macromolecular components of a cell or an organism which a drug molecule has	Carriers activated by a drug	Active macromolecular components of a cell or an organism which a drug molecule has to combine with in

				to combine with in order to elicit its specific effect		order to elicit its specific effect
58	What does “affinity” mean?	A measure of how tightly a drug binds to plasma proteins	A measure of how tightly a drug binds to a receptor	A measure of inhibiting potency of a drug	A measure of bioavailability of a drug	A measure of how tightly a drug binds to a receptor
59	A measure of bioavailability of a drug	A measure of how tightly a drug binds to a receptor	An agonist is a substance that:	Interacts with the receptor without producing any effect	Interacts with the receptor and initiates changes in cell function, producing various effects	Increases concentration of another substance to produce effect
60	Expansion of FISH is	Fluorescent insitu hybridisation	Fluorescent intrinsic hybridisation	Fluorescent intracellular hybridisation	Flurescent intensive hybridisation	Fluorescent insitu hybridisation

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COIMBATORE – 641 021

M.Sc. DEGREE EXAMINATION, AUGUST 2018

DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST – THIRD SEMESTER

MARINE MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date / Session:

Part A

Multiple Choice Questions: No 1 to 20 **20x1 = 20 marks**

1) Attachment of small particles or molecules to a larger particle by electric charge is called as

A. Adsortion B. absortion

C. Fixation D. Attachment

2) _____ is derived from an environment other than that in which it is found.

A. Autothonous B. Allocthonous

C. Heterothonous D. Xenothonous

3) _____ are organism which grow at high pressure rather than at atmospheric pressure.

A. Barophile B. Halophile

C. Thermophile D. Neutrophile

4) TVC means _____

A. Total Viable Count B. Total Non-Viable Count

C. Time Variable Count D. Time Non-Variable Count

5) _____ is the mass of living matter present.

A. Biodivert B. Biogroup

C. Biomass D. Bioaccumulation

6) Agar slants are covered with _____ in culture preservation.

A. oil B. Parafin

C. Grease D. Both a and b

7) The concept of putting microorganism to help clean up the environment is called

A. Bioremediation B. Pasteurization

C. Fermentation D. Biolistics

8) Who is the father of marine microbiology?

A.Louis Pasteur B. Zobell

C. Anton von Leewenhook D. Robert Koch

9) Prokaryotic ribosomes are made up of _____ sub units

A. Two B. Five

C. Three D. Four

10) Bacteria having no flagella are unable to _____

A. Move B. Reproduce

C. Ferment D. Store

11) Viable count method is used to count

A. Only Viable cells B. Only Dead Cells

C. Both a and b D. None of these

12) Expansion of RFLPs

A. Restriction Fragment Length Polymorphism B. Restriction Filament Length Polymorphism

C. Rapid Fragment Length Polymorphism D. Rapid Filament Length Polymorphism

13) Coliforms are used as indictor organism because

A. They are absent wherever enteric organism are present B. a testing procedure with great specificity is easy to perform

C. No change D. they ar present every where

14) Bacteria deficient of cell wall is

A. Treponema B. Mycoplasma

C. Staphylococcus D. Bacillus

15) Single celled bacteria will form a _____ colony

A. Multiple B. Single

C. No D. Infinite

16) Oligonucleotide means containing

A. No Nucleotide B. 10 Nucleotide

C. More than 10 Nucleotide D. Less than 10 Nucleotide

17) BGA expanded as

A. Blue Green Algae B. Blue Grown Algae

C. Brown Green Algae D. Blue Non Grown Algae

18) Xenobiotics are also called as

A. Recalcitrants B. Detoxicants

C. Toxicants D. None of the above

19) Majority of Hydrocarbons found on earth are

A. Crude oils B. Paraafin

C. Water D. Petroleum

20) The bacteria most often involved in spoilage of fish are

A. Sarcina

B. Micrococcus or Bacillus sp.

C. Molds or Yeast

D. Virus

Part B

Answer all the questions **3x2 = 6 marks**

21) Write the Enumeration techniques of Marine Microorganisms?
22) What are Extremophiles?
23) What are the uses of RFLP and RAPD techniques

Part C

Answer all the questions **3x8 = 24 marks**

24) a) Write in detail about the collection of Marine Microorganisms in benthic region?

(or)

b) Write notes on international and national collection centres?

25) a) Write short notes on thermophiles, basophiles, halophiles and psychrophiles?

(or)

b) Brief account on RAPD and RFLP techniques?

26) a) Write notes on degration of biosurfactants?

(or)

b) write notes on degradation of hydrocarbons?

Reg. No. : ----------

[18MBP105A]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
SECOND INTERNAL ASSESSMENT, OCTOBER 2018
FIRST SEMESTER
MICROBIOLOGY
MARINE MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**
Date: /10/2018 **Class: I M.Sc. MB**

PART A – (20 x 1 = 20 marks)

Answer all the questions

1. Which is most likely to be exposed on the surface of a gram-negative bacterium?
a) Pore protein (porin) b) Protein involved in energy generation
c) Lipoteichoic acid d) Phospholipids
2. The arrangement, in which flagella are distributed all round the bacterial cell, is known as
a) Lophotrichous b) Amphitrichous
c) Peritrichous d) Monotrichous
3. Which route of drug administration is most likely to lead to the first-pass effect?
a) Sublingual b) Oral
c) Intravenous d) Intramuscular
4. Which organ involved in first pass effect?
a) Heart b) Kidney
c) Brain d) Liver
5. Which one of the following is not a route of administration?
a) Intravenous b) Oral
c) Topical d) Dissolution
6. Which of the following processes proceeds in the second phase of biotransformation?
a) Acetylation b) Reduction
c) Oxidation d) Hydrolysis
7. An agonist can produce submaximal effects and has moderate efficacy it's called:
a) Partial agonist b) Antagonist
c) Agonist-antagonist c) Full agonist
8. What term is used to describe a decrease in responsiveness to a drug which develops in a few minutes?
a) Refractoriness b) Cumulative effect
c) Tolerance d) Tachyphylaxis
9. Basic drugs are absorbed in
a) Small intestine b) stomach
c) Large intestine d) Pancreas
10) Coenzyme required by Cytochorome p450 MO is
a) NADH b) NADPH
c) Lipoic acid d) TPP
11) Which drug that cross the lipid bilayer easily
a) Water soluble drug b) lipid soluble drug

c) ion soluble drug c) Non ion soluble drug

12) Science that deals with drug

a) Pharmacy b) Pharmacognosy

c) Pharmacodynamics d**)** Pharmacology

13. The stabilizing agents used in cryopreservation is _____

a) Glycerol b) Phenol

c) Terpenol d) Lysol

14. Acidic drug rapidly absorbed at

a) Stomach b) GI tract

c) Large intestine d) Mouth

15. Pick out the parenteral route of medicinal agent administration:

a) Rectal b) Oral

c) Sublingual d) Inhalation

16. Biotransformation of the drugs is to render them:

a) Less ionized b) More pharmacologically active

c) More lipid soluble d) Less lipid soluble

17. Aspirin is chemically

a) Sodium salicylate b) Acetylsalicylic acid

c) Salicylamide d) Sodium salicylamide

18. Irreversible interaction of an antagonist with a receptor is due to:

a) Ionic bonds b) Hydrogen bonds

c) Covalent bonds d) Weak bonds

19. Chloramphenicol is derived from

a) Streptomyces venezulae b) Streptomyces griseus

c) Streptomyces kanamycin d) Pencillin

20. Drug used for the treatment of acute cocaine overdose is

a) Naproxen b) amphetamine

c) diazepam d) Ibu brufen

PART B – (03 x 02 = 06 marks)

Answer all Questions

21. What is the role of microbes in marine environment?

22. Define Photosynthetic pigments and name some of the accessory pigments?

23. Define Cryopreservation?

PART C – (3 x 08 = 24 marks)

Answer all questions choosing either a (or) b. (All questions carry equal marks)

24. a) Explain in detail about Marine Nutrient Cycle? (OR)

b) What are the impacts of Marine Biodiversity?

25. a) Explain in detail about Coral reefs?(OR)

b) What are the differences between Red tides and zones?

26. a) Explain Genome Sequencing and Physical Mapping?

(OR)

b) Explain Cryopreservation?