Journal of Microbiology (2015)
DOI 10.1007/s12275-015-4138-0

Spectral characterization of a pteridine derivative from cyanide-utilizing bacterium *Bacillus subtilis* - JN989651

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(Received Mar 4, 2014 / Revised Nov 27, 2014 / Accepted Nov 28, 2014)

Soil and water samples were collected from various regions of SIPCOT and nearby Vanappadi Lake, Ranipet, Tamilnadu, India. Based on their colony morphology and their stability during subculturing, 72 bacteria were isolated, of which 14 isolates were actinomycetes. Preliminary selection was carried out to exploit the ability of the microorganisms to utilize sodium cyanate as nitrogen source. Those organisms that were able to utilize cyanate were subjected to secondary screening viz., utilization of sodium cyanide as the nitrogen source. The oxygenolytic cleavage of cyanide is dependent on cyanide monooxygenase which obligately requires pterin cofactor for its activity. Based on this, the organisms capable of utilizing sodium cyanide were tested for the presence of pterin. Thin layer chromatography (TLC) of the cell extracts using n-butanol: 5 N glacial acetic acid (4:1) revealed that 10 out of 12 organisms that were able to utilize cyanide had the pterin-related blue fluorescent compound in the cell extract. The cell extracts of these 10 organisms were subjected to high performance thin layer chromatography (HPTLC) for further confirmation using a pterin standard. Based on the incubation period, cell biomass yield, peak height and area, strain VPW3 was selected and was identified as *Bacillus subtilis*. The Rf value of the cell extract was 0.73 which was consistent with the 0.74 Rf value of the pterin standard when scanned at 254 nm. The compound was extracted and purified by preparative High Performance Liquid Chromatography (HPLC). Characterization of the compound was performed by ultraviolet spectrum, fluorescence spectrum, Electrospray Ionization-Mass Spectrometry (ESI-MS), and Nuclear Magnetic Resonance spectroscopy (NMR). The compound is proposed to be 6-propionyl pterin (2-amino-6-propionyl-3*H*-pteridin-4-one).

Keywords: cyanide monooxygenase, *Bacillus subtilis*, HPLC, ESI-MS, NMR, 6-propionyl pterin

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Introduction

Pteridines are heterocyclic nitrogen compounds that are metabolically important as cofactors of enzymes associated with growth and differentiation (Chapman, 1969) viz., aromatic amino acid (phenylalanine, tyrosine, and tryptophan) hydroxylases (AAH), nitric oxide synthases (NOS) and cyanide monooxygenase (CNO). The redox potential of some pteridines indicates that they play a key role in cellular electron transport (Rembold, 1975). Pterin compounds are broadly classified into 2 major classes, 'conjugated' and 'unconjugated'. The classification is based on the complexity of the side chains. Folic acid and methanopterin belong to the conjugated type, which has a linkage of p-aminobenzoic acid to pterin. Biopterin, molybdopterin and pterin-containing glycosides belong to the unconjugated type since they bear less complex side chains at the 6-position of the pterin (Cho *et al*., 1998).

Pterin derivatives were found to be produced in abundance by certain prokaryotes, especially cyanobacteria (Forrest *et al*., 1958; Hatfield *et al*., 1961; Forrest and Van Baalen, 1970; Matsunaga *et al*., 1993). A high concentration of pterins (2-amino 4-hydroxy pteridines) was first reported in *Anacystis nidulans* (Forrest *et al*., 1957). *Synechococcus* sp. and *Spirulina platensis* are the other cyanobacteria that produce pterin derivatives. Anaerobic photosynthetic bacteria *Chlorobium tepidum* (Cha *et al*., 1995), *Chlorobium limicola* (Cho *et al*., 1998), a chemoautotrophic archaebacterium *Sulfolobus solfataricus* (Lin and White, 1988), and a methanogenic bacterium *Methanoculleus thermophilus* were found to contain pterin glycosides. The functions of parent biopterin have been studied in detail but the physiological roles of other forms remain obscure.

Cyanide is a potent poison that arises in the environment by natural and anthropogenic means. Cyanide is highly toxic to living organisms since it inactivates the respiration system by tightly binding to cytochrome C oxidase (Solomonson and Spehar, 1981; Chena and Liu, 1999; Yanase *et al*., 2000). Microbial degradation of cyanide involves enzymatic pathways and generally these are induced by the presence of cyanide in the medium. The oxidative pathway of cyanide conversion involves oxygenolytic conversion to carbon dioxide and ammonia. The oxidative pathway involves cyanide mono-