ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 3, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

ANTI-QUORUM SENSING POTENTIAL OF *LIMONIA ACIDISSIMA (L.)* AGAINST *VIBRIO HARVEYI* KUMB-VA4

SRINIVASAN P[1]*, RAJALAKSHMI M[2], DINESH KUMAR S[1]

[1]Aquaculture and Molecular Microbiology Laboratory, Department of Microbiology, Karpagam Academy of Higher Education (Deemed to be University), Coimbatore - 641 021, Tamil Nadu, India. [2]Department of Bioscience and Research, Shri Nehru Maha Vidyalaya College of Arts and Science, Coimbatore - 641 050, Tamil Nadu, India. Email: srini2k8@gmail.com

Received: 02 November 2017, Revised and Accepted: 09 December 2017

ABSTRACT

Objective: This study aims to investigate the quorum-sensing inhibition (QSI) potential of *Limonia acidissima* L. against the biofilm forming *Vibrio harveyi* isolated from freshwater fish.

Methods: The present study evaluated the anti-QS activity of the *L. acidissima* methanol and ethyl acetate (LA-M and LA-EA) fruit extracts using *Chromobacterium violaceum* ATCC 12472 (wild) and *C. violaceum* CV026 (mutant) as biomonitor strains and biofilm formation using the crystal violet assay. *Vibrio* sp. were isolated from freshwater-cultured fishes and screened for biofilm formation property. Strong biofilm forming isolate were subjected to molecular characterization. *Limonia* fruit pulp was subjected to methanol and ethyl acetate extraction using cold percolation method and yield was calculated. In parallel to determining the QSI properties of the extract, minimum inhibitory concentration (MIC), biofilm inhibition concentration (BIC), antibiofilm properties, and metabolic activity of LA-M and LA-EA against the biofilm forming *V. harveyi* KUMB-VA4 was determined.

Results: The results of the present study demonstrated that the overall yield of methanol and ethyl acetate extract was 12.84% and 9.3% (w/w), respectively. Strong biofilm forming *Vibrio* isolate KUMB-VA4 was obtained from infected freshwater fishes and was subjected to molecular characterization. MIC of LA-M was 1510 µg/ml and LA-EA was observed to be 3000 µg/ml against the test pathogen, respectively. Biofilm inhibition assay revealed a BIC of LA-M at 250 µg/ml and LA-EA at 500 µg/ml. Both the plant extracts significantly reduced the biofilm formation of *V. harveyi* KUMB-VA4 and the metabolic activity in a dose-dependent manner. Light microscopy and scanning electron microscopy revealed that LA-M and LA-EA significantly altered 68.6% and 54.5% of the biofilm architecture at BIC. The QSI assay revealed that LA-M effectively reduced the violacein production of the biomonitor strains at sub-BIC (100–500 µg/ml) to 80% than LA-EA (43%) in a strong dose-dependent fashion.

Conclusions: The present study revealed the QSI property of *Limonia acidissima* against the biofilm forming *V. harveyi* isolated from infected fish.

Keywords: *Limonia acidissima*, *Vibrio harveyi*, *Chromobacterium violaceum*, Quorum-sensing inhibition.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i3.23448

INTRODUCTION

The genus *Vibrio* and their closely related species are significant pathogens affecting the intensive rearing of finfish, molluscs, and shrimps [1]. Traditionally, antibiotics are used to control bacterial diseases in aquaculture sector. However, the frequent application, in many cases even as preventive measures, has resulted in the development and spread of resistance among the bacterial pathogens [2]. *Vibrios* are Gram-negative, rod-shaped bacteria, but they may also be curved or comma-shaped. They are non-sporulating, non-capsulated, facultative anaerobes, catalase-positive, and motile by means of a single polar flagellum [3]. In fish, the diseases include vasculitis, gastroenteritis, and eye lesions. With shrimp, the pathogen is associated with luminous vibriosis. Clinical signs of vibriosis are hemorrhage to intestines, body cavity, spleen and muscle, distended mucoid and necrotic intestine and petechiation, erosion and darkened coloration to the skin and fins. Changes to the eyes include distension and cloudiness and periorbital swelling. White/gray lesions can be found on the intestines and spleen and in fry, splenomegaly.

Decades of research has enabled the recognition of bacterial biofilms as the predominant bacterial survival tactics [4]. Biofilms play a vital role in the fouling process [5] and are known to have astounding rate of tenacious antibiotic resistance [6], one of the dominant obstacles in the antimicrobial chemotherapy. The pathogenicity mechanisms of *Vibrio* are imprecisely understood, with likely mechanisms involving the ability to attach and form biofilms, quorum sensing (QS), various extracellular products including proteases and hemolysins, lipopolysaccharide, and interaction with bacteriophage and bacteriocin-like substances [7]. QS, or bacterial cell-to-cell communication, is a key process for bacterial colonization of substrata through biofilm formation, infections, and production of virulence factors. It involves the regulation of coordinated behaviors in bacteria as a function of population density which is achieved by the production, excretion, and detection of autoinducer (AI) molecules [8]. These AI molecules trigger the QS mechanism which aids the bacteria to optimize their energy metabolism and regulate its characteristic functions such as biofilm formation and expression of virulence factors. Biofilm formation by *Vibrio* sp. enhance the development of antibiotic resistance and sometimes the synthesis of bioluminescence pigment [9]. With the rapid developments in aquaculture, particularly in Asia, the organism has become a serious cause of disease in fish. Study on the QS inhibitors (QSIs) has gained prominence as the QSIs do not exert a selective pressure on the bacterial population and growth thereby successfully avoid the development of resistance toward antibiotic treatments [10]. Moreover, reports elucidate that QS signaling molecules interact directly with the biofilm forming bacterial colonies [11] and so QS disruption, directly or indirectly prevents the biofilm formation on underwater surfaces and