UNIVERSITI PUTRA MALAYSIA

EFFECTS OF BACTERIAL QUORUM SENSING DEGRADER ON Artemia franciscana CULTURE

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FACULTY OF AGRICULTURE

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CERTIFICATION OF APPROVAL

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This is to certify that I have examined the final project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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ABSTRACT

Quorum sensing is a bacterial cell-to-cell communication with small signal molecules such as acyl-homoserine lactones (AHL) that can regulates the virulence of many pathogenic bacteria. Therefore, quorum sensing interference using degrader strains is a biocontrol strategy to fight bacterial infections. In this study, one of the quorum sensing degrader strains, BP-ART/6 was successfully isolated from *Artemia franciscana* by enriching newly hatched *Artemia* nauplii in AHL. Anti-quorum sensing activity of BP-ART/6 was screened using *Chromobacterium violaceum* CV026 biosensor bioassay, and having the best degradation properties which fully degrade 10ppm of AHL in less than 24 hours. The Gram staining test indicated BP-ART/6 as Gram-negative bacteria. Strain BP-ART/6 was resistant to antibiotic kanamycin and rifampicin at 20, 50 and 100ppm, respectively after 24 hours and 48 hours of incubation. Result from antibacterial study showed BP-ART/6 has no antibacterial activity against *Vibrio campbellii* ATCC14126, *Vibrio alginolyticus* ATCC17749 and *Vibrio anguillarum* ATCC43313. Significant differences ($p < 0.05$) of survivability in the presence of the degrader strain can be observed between control and the treatment with the QS degraders at both 10^5 and 10^6 CFU/ml for 6, 12 and 24 hours. At the same time, encapsulation of degrader strains BP-ART/6 by *Artemia* was less than 30 colonies as the bacterial counts lay below the countable range of 30 to 300 colonies.

ABSTRAK

Kuorum penginderaan merupakan komunikasi antara sel bacteria dengan molekul kecil iaitu N-acy-homoserine lakton (AHL) yang boleh mengawal kebisaan pelbagai bakteria berpatogenik. Oleh itu, penggunaan strain degradsi dalam gangguan kuorum penginderaan merupakan strategi kawalan biologi untuk menentang jangkitan bacteria. Salah satu strain degradsi, BP-ART/6, telah berjaya diasingkan melalui kaedah pengayaan daripada *Artemia franciscana* yang baru menetas.Aktiviti anti-Kuorum penginderaan BP-ART/6 telah diuji menggunakan *Chromobacterium violaceum* CV026 sebagai biosensor bioesei, and mempunyai ciri-ciri degradasi yang baik yang boleh mendegradkan 10ppm AHL dalam masa kurang daripada 24 jam. Ujian Gram stain menunjukkan BP-ART/6 sebagai bakteria Gram-negatif. BP-ART/6 resisten terhadap antibiotic kanamycin and rifampicin pada 20, 50 and 100ppm selepas 24 dan 48 jam inkubasi. BP-ART/6 tidak menunjukkan aktiviti antibakteria terhadap *Vibrio campbellii* ATCC14126, *Vibrio alginolyticus* ATCC17749 and *Vibrio anguillarum* ATCC43313. Perbezaan signifikan ($p < 0.05$) tahap kehidupan dalam kewujudan strain degradasi amat ketara antara kontrol dengan sampel degradasi pada 10^5 and 10^6 CFU/ml untuk 6, 12 and 24 jam. Pada masa yang sama, pengkapsulan strain degradasi oleh *Artemia* adalah rendah disebabkan jumlah kiraan bacteria adalah kurang daripada 30 hingga 300 koloni.

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%	Percent
˚C	Degree Celsius
≈	Approximate
>	Greater than
<	Less than
rpm	Round per minute
ppm	Parts per million
US$	United States dollar
min	Minutes
x	Times
M	Molar
CFU	Colony-forming unit
g	Gram
kg	Kilogram
mm	Millimetre
cm	Centimetre
ml	milliliter
L	Liter
µm	Micrometre
µl	Microliter

CHAPTER 1

INTRODUCTION

Quorum sensing (QS) is a mechanism in which bacteria are able to coordinate the expression of certain genes in response to the presence of small signal molecules (Hense *et al.*, 2007). It is a type of bacterial cell-cell communication that can regulate the expression of virulence genes in many pathogenic bacteria, and most of them usually use different signal molecules to regulate virulence gene expression (Pande *et al*., 2013). Bassler (2002) stated that quorum sensing can regulates bioluminescence, virulence factor expression, biofilm formation, sporulation, and mating. According to Natrah (2011b), aquaculture pathogens also use QS to regulate the expression of important virulence phenotypes. High mortality of larvae is observed in *Macrobrachium rosenbergii* (Baruah *et al.*, 2008) and burbot larvae (Natrah *et al.*, 2012) in the presence of pathogen with QS activity.

Fish larvae often undergo heavy mortalities in early stage which is four to nine days after hatching (Kohno *et al.*, 1997), and often exposed to Vibrio diseases such as *Vibrio alginolyticus, Vibrio harveyi, Vibrio vulnificus* and other *Vibrio* spp. (Huang *et al*., 2012). Among the preventive measures are through the use of antibiotic. However, the frequent use of antibiotic treatment has resulted in the development of antibiotic resistance, causing this compound to be ineffective to control vibriosis (Defoirdt *et al.*, 2007). Since QS is important for the virulence

towards aquatic host, QS degrader could also be an alternative solution for disease control (Defoirdt *et al*., 2013). Hence, QS degrader strain can be inculcated into live feed as a carrier.

Live prey such as *Artemia* nauplii have successively been used to rear marine fish larvae because it is high in free amino acids with small peptides, or other water-soluble nutrients (Johnson *et al.*, 2009). The research also stated *Artemia* having consistent availability and quality which increased nutrient delivery and addition of probiotic can support the larval growth of a particular species. Hence, the objectives of this research are:

1. To isolate quorum sensing degrader from *Artemia*
2. To test the quorum sensing degrader encapsulated to *Artemia*

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