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High-throughput Screening for Quorum Sensing-inhibitory Compounds from Selected Philippine Marine Algae and Surface-associated Marine Microorganisms for Potential Anti-biofilm/biofouling Applications

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One of the main global problems that cause significant losses in mariculture, medical, and industrial fields is biofilm formation and biofouling (Bixler and Bhushan 2012). Biofilms, which are composed of thick layers of cells embedded in an intricate exopolysaccharide matrix, are often the preferred mode of growth for most bacteria. Biofilms manifest as the slime often found attached to surfaces in aquatic or marine environments, or even in medical polymers such as catheters and implants. Biofilm growth provides a strategic niche for planktonic bacteria to thrive in environments prone to mechanical and chemical disruptions. In the complex domain of the extracellular carbohydrate matrix of biofilms, bacterial aggregates become much less susceptible to treatment with the most diverse chemical biocides and antibiotics than the planktonic cells (Joint et al. 2007). It has been shown that bacteria and biofilms are able to act together concertedly through the metabolism of compounds formed within the intact community of bacterial biofilm cells. These compounds were then later associated with quorum sensing (QS) (Rasmussen and

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Givskov 2006). The formation of biofilms, which lead to biofouling or the development of complex biological communities on surfaces, such as ship hulls and aquaculture substrates, results in massive material and economic loss (Lehaitre et al. 2008). In different ecological environments, nature has given advantage to organisms that are able to diminish or eradicate detrimental biofouling.

Quorum sensing is defined as a community genetic regulation mechanism that controls microbiological functions of medical, agricultural, and industrial importance in response to population density (Zhang and Dong 2004). Concomitant to an increase in bacterial population, small, freely diffusible signal molecules excreted by the organisms will accumulate. A critical threshold concentration is necessary to initiate a response in the whole population and to activate target genes essential in the synchronization of gene expression and functional coordination among bacteria (Swift et al. 2001; Zhang and Dong 2004).

Studies show that some human and plant pathogens, such as *Pseudomonas aeruginosa* and *Erwinia carotovora*, regulate virulence through quorum signaling. Quorum sensing has been suggested as an ideal target for treatment of both Gram-negative and Gram-positive bacterial infections often associated with the formation of biofilms (Kim et al. 2007). Interception of this communication pathway thus implies a potential for QS inhibition to prevent diseases and adverse environmental problems, such as biofilm growth and biofouling.

QS-inhibitory molecules that were initially identified include triclosan, furanone, 3-oxo-C12-(2aminocyclohexanone), furanylhydrazides and macrolides, 2-O-glycerol-*alpha*-D-galactopyranoside (floridoside), betoncine, isethionic acid, and several other molecules (Zhang and Dong 2004). The red alga *Delisea pulchra* was shown to produce a class of halogenated furanones that accelerate the turnover of the N-acyl homoserine lactone (AHL)-responsive regulatory protein of the LuxR family and inhibit bacterial quorum sensing and biofilm formation (Manefield et al. 2002). On the other hand, the green alga *Ulva lactuca* was demonstrated to rely on the epiphytic bacterium *Pseudoalteromonas tunicata* to inhibit AHL-dependent transcriptional control by means of synthesizing pigmented substances (Egan et al. 2002).

There are numerous studies worldwide reporting the bioactivity of marine-sourced organisms, and these provide impetus to utilize the rich biodiversity of the relatively untapped seaweed resources of the Philippines. This work utilized a high-throughput screening method for quorum sensing-inhibitory molecules from selected Philippine marine algae and associated marine microorganisms. This will subsequently aid in the systematic fast identification, extraction, and upscale

production of the bioactive molecules for applications in the prevention of biofilm formation and biofouling, especially in mariculture systems.

Seaweed samples were obtained from 36 sites around Luzon, Visayas, and Mindanao. Out of 86 seaweed fragments collected from the respective sampling sites, six yielded positive results for QSI. Of the 179 microbial isolates that were assayed, nine exhibited QS inhibitory activity.

A total of 51 crude methanol extracts from seaweed were obtained. A total of 25 out of 51 crude seaweed extracts tested positive for QS inhibitory activity (Table 1). Solvent fractions for the crude methanol extracts of *Hydropuntia edulis, Halymenia durvillei, Chaetomorpha crassa,* and *Halimeda macroloba* were also tested using agar well diffusion assay (Table 2). The putative QS inhibitor(s) in *H. edulis* possesses non-polar to partially non-polar characteristics, similar to that of *H. durvillei*. Comparison of the size of the pigment inhibition in *H. edulis* shows more pronounced QSI activity in the hexane partition compared to the ethyl acetate partition. The putative QS inhibitors in both *C. crassa* and *H. macroloba* are partially polar.

Table 1. Seaweed extract positive for QSI

Seaweed	Site	Collection Period
Halymenia durvillei	Bolinao, Pangasinan	October 2015
Chaetomorpha crassa	Balaoan, La Union	June 2014
Gracilaria sp.	Buguey, Cagayan	June 2014
Halimeda opuntia	Santa Ana, Cagayan	June 2014
Tricleocarpa fragilis (=*Galaxaura oblongata*)	Santa Ana, Cagayan	June 2014
Padina sp.	Santa Ana, Cagayan	June 2014
Jania sagittata (=*Cheilosporum sagittatum*)	Santa Ana, Cagayan	June 2014
Halimeda macroloba	Gonzaga, Cagayan	June 2014
Halimeda opuntia	Gonzaga, Cagayan	June 2014
Hormohysa cuneiformis	Gonzaga, Cagayan	June 2014
Kappaphycus cottonii	Calatagan, Batangas	April 2015
Hydropuntia edulis	Calatagan, Batangas	October 2015
Mastophora rosea	Lapu-lapu, Cebu	September 2014
Ulva reticulata	Lapu-lapu, Cebu	September 2014
Turbinaria ornata	Moalboal, Cebu	September 2014
Amphiroa foliacea	Moalboal, Cebu	September 2014
Chondrophycus cartilagineus (=*Laurencia cartilaginea*)	Oslob, Cebu	September 2014
Padina sp.	Oslob, Cebu	September 2014
Padina sp.	Panglao, Bohol	September 2014
Halimeda macroloba	Panglao, Bohol	September 2014
Galaxaura divaricata (=*Galaxaura fasciculata*)	Panglao, Bohol	September 2014
Halimeda macroloba	Loon, Bohol	September 2014
Mastophora rosea	Loon, Bohol	September 2014
Ulva reticulata	Jagna, Bohol	September 2014
Chondrophycus tronoi (=*Laurencia tronoi*)	Sarangani Island, Davao Occidental	February 2016

Table 2. QSI activity of seaweed partitions

Seaweed Species	Crude extract	Partition		
		Hexane	Ethyl Acetate	Aqueous
Chaetomorpha crassa	+	-	+	-
Hydropuntia edulis	+	+	+	-
Halimeda macroloba	+	-	+	-
Halymenia durvillei	+	+	+	-

The method described by Chaudhari et al. (2014) and Choo et al. (2006) quantifies changes in violacein production and cell density according to the addition of seaweed and microbial extracts. The results obtained using this method provide more definitive data for QS inhibitory activity. Inhibition of purple pigmentation with constant cell density indicated QSI. On the other hand, a decrease in optical density with diminished pigmentation is attributed to cell death, which often implies antibacterial activity and not QSI.

The crude extract of *H. edulis* has a lower absorbance reading for violacein compared to the negative control (methanol). There is a 75.6% decrease in the violacein absorbance in the crude *H. edulis* extract compared to the negative control. The cell density remained relatively constant, with a decrease of only 14.5%, similar to the results obtained using the positive control cinnamaldehyde. The decrease in QS-regulated violacein production without manifestations of bacterial death confirms the QS-inhibitory activity of the crude *H. edulis* extract. A Tukey post-hoc test confirmed that, while the difference in violacein between methanol and crude *H. edulis* extract is significant (p= 5.801E-09), the difference in cell density is not (p=.011).

The *in-situ* method provides a rapid and systematic means of screening for QS inhibition exhibited by seaweeds and associated marine microorganisms in the field. The agar well diffusion assay, although quick and convenient, has several limiting factors in terms of miscibility, volume, and molecule size of the possible QS inhibitors. On the other hand, the broth assay provides more quantitative information on the relative absorbance range of potential bioactive compounds. This further facilitates the isolation and characterization process, and validates the results obtained in the agar well diffusion assay. Overall, the three assays described provide high throughput methods to maximize the screening of available QS inhibitory compounds from marine seaweeds and associated microorganisms.

SUPPLEMENTARY MATERIAL

Experimental details relating to this paper are available online: http://www.journals.upd.edu.ph/index.php/sciencediliman/article/view/5626/5046

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REFERENCES

Bixler GD and Bhushan B. 2012. Biofouling: Lessons from nature. Philosophical Transactions of the Royal Society. 370:2381-2417.

Chaudhari V, Gosai H, Raval S, Kothari V. 2014. Effect of certain natural products and organic solvents on quorum sensing in *Chromobacterium violaceum*. Asian Pacific Journal Tropical Biomedicine. 4:S890-S897.

Choo JH, Rukayadi Y, Hwang JK. 2006. Inhibition of bacterial quorum sensing by vanilla extract. Letters in Applied Microbiology. 42:637–641.

Egan S, James S, Kjelleberg S. 2002. Identification and characterization of a putative regulator controlling the expression of fouling inhibitors in *Pseudoalteromonas tunicate*. Applied & Environmental Microbiology. 68:372-378.

Joint I, Tait K, Wheeler G. 2007. Cross-kingdom signalling: Exploitation of bacterial quorum sensing molecules by the green seaweed *Ulva*. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 362:1223-1233.

Kim J, Kim Y, Seo Y, Park S. 2007. Quorum sensing inhibitors from the red alga *Ahnfeltiopsis flabelliformis*.Biotechnology and Bioprocess Engineering. 12:308-311.

Lehaitre M, Delauney L, Compere C. Biofouling and underwater measurements. In: Babin M, Roesler CS, Cullen JJ, editors. Real-Time Coastal Observing Systems for Marine Ecosystem Dynamics and Harmful Algal Blooms: Theory, instrumentation and modelling. Oceanographic Methodology series. Paris: Unesco Publishing; 2008. p. 463-493.

Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, Kjelleberg S, Givskov

McLean RJ, Pierson LS, Fuqua C. 2004. A simple screening protocol for the identification of quorum signal antagonists. Journal of Microbiological Methods. 58:351–360.

Rasmussen TB, Bjarnsholt T, Skindersoe ME, Hentzer M, Kristoffersen P, Kote M, Nielsen J, Eberl L, Givskov M. 2005. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. Journal of Bacteriology. 187(5):1799–1814.

Rasmussen TB, Givskov M. 2006. Quorum sensing inhibitors: A bargain of effects. Microbiology. 152:895-904.

Swift S, Downie JA, Whithead N, Barnard AML, Salmond GPC, Williams P. 2001. Quorum sensing population density dependent determination of bacterial physiology. Advances in Microbial Physiology. 45:199270.

Zhang LH and Dong YH. 2004. Quorum sensing and signal interference: diverse implications. Molecular Microbiology. 53:1 563-1571.

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