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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

Supplementary Material

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FIELD COLLECTION OF SAMPLES AND *IN-SITU* DETECTION OF QUORUM SENSING INHIBITION

Seaweed samples were obtained from 36 sites around Luzon, Visayas, and Mindanao (Table 1). Necessary permits were obtained from relevant government units in coordination with the regional off ices of the Bureau of Fisheries and Aquatic Resources (BFAR). Since a greater variety of seaweed species is observed during specific months, sampling visits accounted for the seasonality of seaweeds. Voucher specimens were submitted to the GT Velasquez Herbarium at the UP Marine Science Institute for proper identification and future reference.

Table 1. Field collection sites

Site	GPS Coordinates/Municipality
Luzon	
Poblacion Dos, Calatagan, Batangas	13°49'47.0"N 120°37'02.9"E
San Antonio, Zambales	14°54'50.6"N 120°00'29.7"E
Minanga Weste, Buguey, Cagayan	18°16'5.69"N 121°52'1.77"E
Minanga Weste, Buguey, Cagayan	18°16'9.82"N 121°51'39.12"E
Gonzaga, Cagayan	18°22'48.73"N 122° 5'57.62"E
Santa Ana, Cagayan	18°22'48.62"N 122° 5'57.97"E
Taggat Sur, Claveria, Cagayan	18°36'44.00"N 121° 2'55.29"E
Sitio Banwa, Balaoi, Pagudpud, Ilocos Norte	18°35'38.46"N 120°53'5.99"E
Casa Teresita, Pagudpud, Ilocos Norte	18°36'29.84"N 120°46'42.55"E
Paayas, Burgos, Ilocos Norte	18°29'56.31"N 120°34'11.04"E
Currimao, Ilocos Sur	17°59'30.47"N 120°29'46.39"E
Sinait, Ilocos Sur	17°51'53.33"N 120°26'25.30"E
Santa Maria, Ilocos Sur	17°22'4.12"N 120°27'2.28"E
Balaoan, La Union	16°48'12.89"N 120°19'42.38"E
Rosario, Ilocos Sur	16°13'0.44"N 120°24'45.66"E
Bolinao, Pangasinan	16°22'40.7"N 119°54'42.0"E

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Table 1. Field collection sites (Cont'n.)

Site	GPS Coordinates/Municipality
Visayas	
Marine Sanctuary, Luyang, Carmen, Cebu	10°35'53.88"N 124° 1'45.30"E
Punta Engaño, Lapu-lapu, Cebu	10°19'51.81"N 124° 2'40.01"E
Moalboal Beach Resort, Saavedra, Moalboal, Cebu	9°59'7.76"N 123°23'5.31"E
Saavedra Fish Sanctuary, Moalboal, Cebu	9°59'51.36"N 123°22'46.79"E
BCD's Pace, Tan-awan, Oslob, Cebu	9°27'46.80"N 123°22'45.91"E
Calaguyan Sur, Loon, Bohol	9°50'32.13"N 123°47'42.03"E
Tambulian Shoal, Tubigon, Bohol	10° 3'20.16"N 123°56'34.17"E
Ubay Island, Tubigon, Bohol	10° 1'21.26"N 123°58'0.57"E
Poblacion, Bien Unido, Bohol	10° 8'10.16"N 124°22'51.06"E
Larapan, Jagna, Bohol	9°39'10.39"N 124°22'51.31"E
Doljo, Panglao, Bohol	9°35'10.27"N 123°43'27.94"E
Mindanao	
Tibungco, Davao City, Davao del Sur	7°11'07.4"N 125°39'17.2"E
Bunawan, Davao City, Davao del Sur	7°14'28.4"N 125°39'10.1"E
Passig Islet, Digos, Davao del Sur	6°47'10.1"N 125°23'42.2"E
Sarangani Island, Davao Occidental	5°25'30.5"N 125°27'44.1"E
Moncado Poblacion, Samal Island, Davao del Norte	7°07'50.2"N 125°40'55.0"E
Miranda Poblacion, Samal Island, Davao del Norte	7°08'19.1"N 125°40'59.8"E
Tambo, Samal Island, Davao del Norte	7°08'57.9"N 125°41'07.7"E
Kawas Beach, Alabel, Saranggani	6°04'00.2"N 125°16'37.2"E
Bula Beach, General Santos City, Saranggani	6°05'58.7"N 125°11'55.7"E

Immediately after the field collection, preliminary qualitative high-throughput screening was performed with the seaweed fragments and microbial isolates using the method described by McLean et al. (2004). This determines whether QS inhibitory compounds are present on the seaweed surface and efficiently tests all surface-associated microorganisms. The bacterial sensor *Chromobacterium violaceum* (CV 12472), which characteristically produces a water-insoluble purple pigment called violacein through the QS network, was used as an indicator strain. Quorum sensing inhibition activity would appear as zones around seaweed fragments or microbial isolates, indicating CV 12472 growths devoid of purple coloration.

Seaweed fragments were cut into smaller size fragments (ca. 1 cm) aseptically and surface-sterilized. The fragments were subsequently washed in sterile seawater and placed on Luria broth-agar (LBA). Surface-associated microbial isolates were streaked on LBA and were incubated for 24 hours. Soft LBA inoculated with an overnight culture of CV 12472 was then poured over the plates with the seaweed fragments and isolates. The plates were observed after 24 hours of incubation.

Results of the *in-situ* assays are depicted in Figure 1. Colorless zones around the seaweed fragment or microbial isolate indicate the presence of potential QS inhibitory compounds.

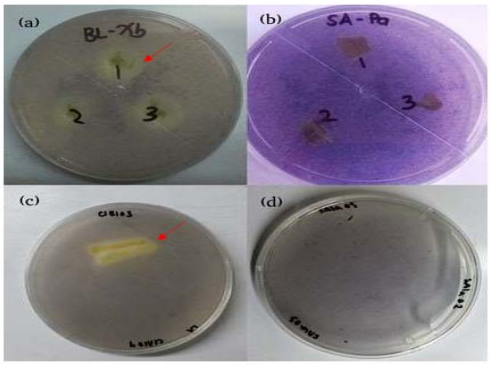

Figure 1. Results of the in-situ assay. (a) Seaweed fragments of *Chaetomorpha crassa* exhibiting QS inhibitory activity (with replicates labelled as 1, 2, 3); (b) non-inhibitory seaweed fragments of *Padina* sp.; (c) microbial isolate showing the decolorization of *Chromobacteria violaceum;* and (d) a non-inhibitory microbial isolate.

CRUDE EXTRACTION AND AGAR WELL DIFFUSION

A total of 51 crude methanol extracts from seaweed were obtained, and percent yields based on dry weight are presented in Figure 2. In order to test for QS inhibitory compounds in seaweed metabolites, all crude extracts were screened for QS inhibitory activity through agar well diffusion assay, similar to the method used by Rasmussen et al. (2005), but with CV12472 as reporter strain.

Wells were bored on layers of LBA and soft LBA inoculated with an overnight culture of CV12472, in order to accommodate 50 ì L of extract. Plates were incubated for 24 hours at room temperature and observed. The absence of the purple pigmentation in areas surrounding the wells signifies the inhibition of quorum sensing. Figure 3 depicts the inhibition of violacein production in CV12472 agar plates by QSI-positive extracts. Figure 3 shows the presence of zones of inhibition for the crude extract, as well as the hexane and ethyl acetate partitions.

QUORUM SENSING INHIBITION ASSAY IN BROTH CULTURES

The extract, together with an overnight culture of *C. violaceum* CV12472 in Luria broth, was incubated at room temperature for 24 hours without agitation. Violacein production and cell density were quantified by measuring the optical density (i.e. absorbance) at 585 nm and 600 nm, respectively. As illustrated in Figure 4, the crude extract of *H. edulis* has a lower absorbance reading for violacein compared to the negative control (methanol).

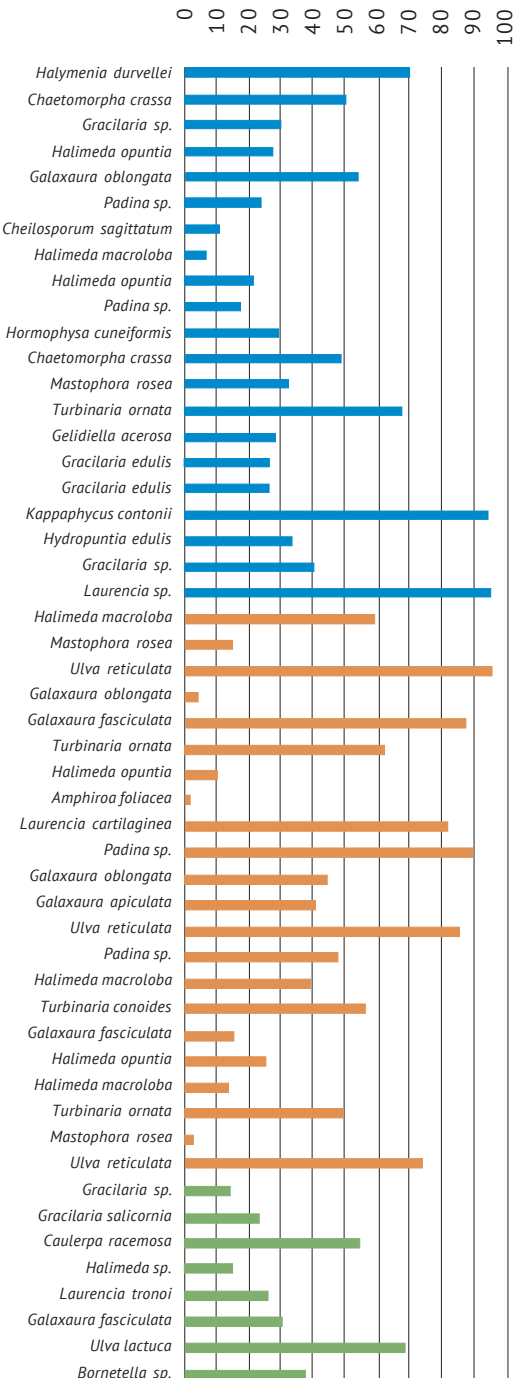

Figure 2. Percent yields (based on dry weight) of crude extracts of seaweed species from Luzon (blue), Visayas (orange), and Mindanao (green).

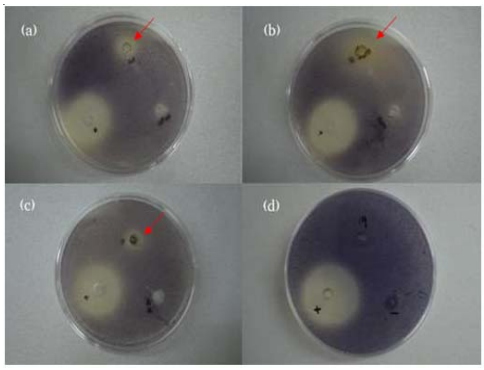

Figure 3. Agar well plate diffusion assay for the (a) crude extract, (b) hexane partition, (c) ethyl acetate partition, and (d) aqueous partition of Hydropuntia edulis. On each plate, the negative control (methanol, hexane, ethyl acetate, and water, repectively) is located on the lower right, while the positive control (75 mM cinnamaldehyde) was placed on the lower left.

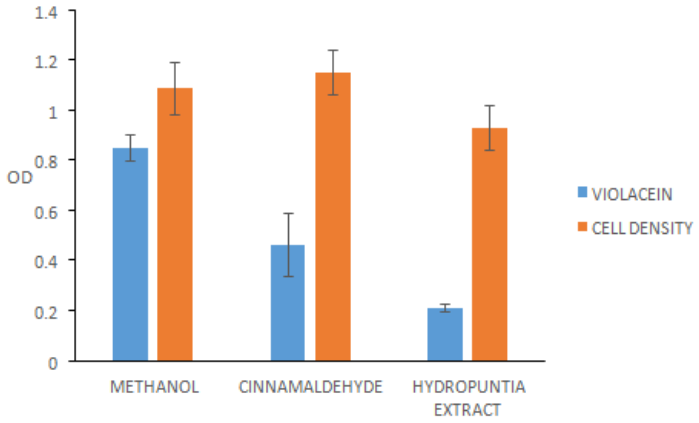

Figure 4. Liquid assay of crude *H. edulis* extract (100 mg/ml), methanol, and cinnamaldehyde (75mM). Methanol and cinnamaldehyde served as the negative and positive controls, respectively. Data presented as mean ± SD (n=6).

REFERENCES

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.