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INTRACELLULAR AND EXTRACELLULAR ANTIFOULING COMPOUNDS FROM THE SEAWEED ASSOCIATED SYMBIOTIC BACTERIAL STRAIN AGAINST BIOFILM BACTERIA

Environmental Science

Siva Selva Rajan A. S R Centre for Scientific Research and Social Resource, 54,4th Street, Ramavarmapuram, Nagercoil, Kanyakumari– 629001, TamilNadu.

ABSTRACT

The antifouling compounds from marine environmental source and for that, the marine macroalgae *Caulerpa prolifera* and its symbionts were selected. The extracellular peptides (toxin) and the ethyl acetate extracts of symbionts were tested for its potential source of ecofriendly antifouling compounds. For the present study, the Seaweed *Caulerpa prolifera* collected from Arokiapuram coast of Kanyakumari District, Tamil Nadu. Then stored aseptically and transferred to the laboratory. The symbiotic bacterial strains belonging to four genera were isolated from the seaweed *C. prolifera*. The bacterial species identified were: *Carnobacterium divergens*, *Alcaligens latus*, *Erythrobacter longus*, *Alcaligens faecalis* and *Azomonas agilis*. Antimicrofouling activity of ethyl acetate extract of symbiotic bacterial strains was determined. Among the tested strains the extracts of *E. longus* and *A. faecalis* exhibited the maximum growth inhibitory activity against biofilm bacteria and the antagonistic activity was ranged from 6mm to 12mm and 6mm to 10mm.The antimicrofouling activity of extracellular compounds of symbiotic bacterial strains such as *E. longus* and *A. faecalis* registered the strong antagonistic activity and it ranged from 6mm to 15mm and 8mm to 15mm against the biofilm bacterial strains.

KEYWORDS

Antifouling compounds, *Caulerpa prolifera, Carnobacterium divergens, Alcaligens latus, Erythrobacter longus, Alcaligens faecalis*

INTRODUCTION

Biofouling or biological fouling is the accumulation of microorganisms, plants, algae or small animals. Such accumulation is referred to as epibiosis when the host surface is another organism and the relationship is not parasitic. Since biofouling can occur almost anywhere water is present, bio fouling poses risks to a wide variety of objects such as boat hulls and equipment, medical devices and membranes, as well as to entire industries, such as paper manufacturing, food processing, underwater construction, and desalination plants. Anti-fouling is the term used to underline the ability of specifically designed materials, such as toxic biocide paints or non-toxic paints to remove or prevent biofouling.

Biofouling organism such as blue mussels, barnacles and macro algae cause serious problems by settling on ships hulls, cooling systems of power plants and aquaculture nets (Characklls *et al*., 1991). Algae impose not only an ecological threat to marine organisms that may be fouled, but are also an economic threat, when fouling artificial substrata such as ships, docks or pipelines (Haderlie, 1984) or mariculture facilities.

In laboratory systems, biofilm bacteria often form highly differentiated three dimensional structures (Microcolonic). Micro colony formation has been demonstrated for most of the model biofilm forming bacteria, e.g. *Escherichia coli*, *Vibrio cholera* and *Pseudomonas aeruginosa* (Davey, *et al*., 2000; Watnick, *et al*., 1999).

Bacteria in biofilms are physiologically and morphologically different from their planktonic growing counter parts. Fossilled biofilms are the first records of life on earth dating back 3.5 billion years and represent by far the most successful form of life, colonizing soils, sediments, mineral and plant surface in nature, including extreme environments, such as pore systems in glaciers, hot vents, electrodes and even highly irradiated areas of nuclear power plants. Self-purification process in nature is performed by biofilm organisms.

The inhibition of fouling from the surface of marine organisms has been a theme in the literature for many years (Sieburth & Conover, 1965; Clare, *et al*., 1996) and a wide array of marine natural products or crude extracts that have some antifouling activity in laboratory bioassays. They have been isolated from a large number of marine organisms, including marine bacteria, seaweeds, sea grasses, bryozoans, ascidians, enidarians and sponges (Rittschof, 2001).

MATERIALS AND METHODS

The isolated symbiotic bacterial strains were screened for antagonistic activity against the biofilm bacteria by following cross streak method. Then the potent symbiotic bacterial strains such as *Carnobacterium divergens, Alcaligens latus, Erythrobacter longus, Alcaligens faecalis* and *Azomonas aglis* were selected for further study. To explore the antimicrofouling activity, the selected symbiotic bacteria were mass cultured individually using Zobell Marine broth (100 ml) in rotatory shaker for 72 hr. Then the culture was mixed with equal volume of ethyl acetate and kept in shaker for 12 hrs. After this, the crude extract was separated using separating funnel and then, the extract was concentrated by evaporation.

The nine biofilm bacterial strains were inoculated in Zobell Marine agar and incubated for 24hrs being used in the antibacterial assay. All the biofilm bacterial strains were seeded individually in the Zobell Marine agar plates. Standard disc diffusion method described by Kirby-Bauer method (Bauer, *et al*., 1966) was followed for the antibacterial assay. The sterile Whatman No.1 filter paper discs of 6mm diameter were impregnated with 20µg /disc of the ethyl acetate extract of symbionts, air dried and placed on the Zobell Marine agar plates and incubated for 18-24hrs at incubation chamber. The assay was carried out in triplicate. Control discs with solvents alone were also maintained. Zone of inhibition was measured from the edge of the disc to the clear zone in millimeter.

The symbiotic bacterial strains were screened against nine biofilm bacteria Microbial culture collections, by following agar double layer method. Then the screened symbiotic bacterial strains were grown aerobically in Zobell Marine broth for 48hrs at 37°C. The antagonistic activity of symbiotic bacterial strains was detected by agar well diffusion assay. Briefly, the stationary phase culture of symbiotic bacterial strains was centrifuged at 2500 × g at 4°C for 10 min. Then the culture supernatant was supernatant was subjected to membrane filtration. From the obtained cell free supernatant, 50µl was placed on the well of Zobell Marine Agar plates which was overlaid by approximately 5ml of soft agar [2.0% agar and 1ml of indicator strain (biofilm bacteria)]. After that, the plates were incubated overnight and then the antimicrofouling activity as zone of inhibition was observed and measured in millimetre.

RESULTS

Antimicrofouling activity of ethyl acetate extract of symbiotic strains

Antimicrofouling activity was carried out with ethyl acetate extract of five symbionts of *C. prolifera* against nine biofilm bacterial strains and the results obtained at shown in Table 1. Among the bacterial symbionts *E. longus* and *A. faecalis* exhibited the maximum growth inhibitory activity than other symbiotic bacterial strains by forming zone of inhibition within the range from 12mm to 15mm and 11mm to 15mm against all the tested biofilm bacterial strains. Next to this, the symbiont *A. aglis* also showed a moderate level of growth inhibition ranged from 11mm to 13mm against at the tested biofilm bacterial strains. The minimum growth inhibitory activity in the range from 8mm to 11mm was expressed by the symbiotic bacterial strain *C. divergens* against all the biofilm bacterial strains. The other two symbiotic extracts exhibited resistance against all the fouling strains (Table 1).

Table 2 provides the result on the antimicrofouling activity of the

extracellular compounds isolated from the symbiotic strains. Among the tested strains, the maximum inhibitory zone was exhibited by *A. latus* than the other symbiotic strains. It formed the zone of inhibition within the range of 9.7mm to 12mm against all biofilm bacterial strains except *A. aquaticus*. The moderate range of inhibitory zone was exhibited by the strain E. longus within the range of 11mm 12mm against all biofilm bacterial strains except *A. aquaticus* Table 2.

Table 1. Antifouling activity (Zone of inhibition – mm level) of ethyl acetate extract of symbiotic strains against biofilm bacterial strains

Biofilm bacterial strains	Symbiotic strains				
	Erythrobacter longus	Alcaligens latus	Alcaligens faecalis	Carnobacterium divergens	Azomonas aglis
Staphylococcuscohnii	6.5 ± 0.40	7.1 ± 0.62	7.8 ± 0.62	6.0 ± 0.20	R
Kurthia gibsonii	R	9.0 ± 0.40	10.1± 0.62	8.1 ± 0.29	7.2 ± 0.43
Micrococcus aglis	7.5 ± 0.40	10.1± 0.62	9.0 ± 0.81	7.0 ± 0.16	R
Renibacterium salmoninarum	R	11.8 ± 0.62	10.1 ± 0.62	10.0 ± 0.40	R
Marinococcus halophilus	R	11.8 ± 0.62	10.1 ± 0.62	6.7 ± 0.29	8.1 ± 0.26
Planococcus citreus	6.5 ± 0.40	11.8 ± 0.62	8.1 ± 0.32	R	R
Halomonas halodurans	R	7.1 ± 0.84	12.0 ± 0.40	11.6 ± 0.29	6.1 ± 1.02
Micrococcus roseus	6.9 ± 0.32	11.0 ± 0.40	6.2 ± 0.43	7.0 ± 0.61	5.8 ± 0.62
Ancyclobacter aquaticus	8.00 ± 0.40	7.5 ± 0.40	R	5.9 ± 0.32	6.5 ± 0.40

R: Resistance.
Each value is the mean ± SD of three estimates.

Table 2. Antifouling activity (Zone of inhibition – mm level) of extracellular bacteriocin of symbiotic strains against biofilm bacterial strains

Biofilm bacterial strains	Symbiotic strains				
	Erythrobacter longus	Alcaligens latus	Alcaligens faecalis	Carnobacterium divergens	Azomonas aglis
Staphylococcus cohnii	8.0 ± 0.40	7.9 ± 0.49	13.6 ± 0.64	10.9 ± 0.32	10.5 ± 0.40
Kurthia gibsonii	R	6.2 ± 0.20	R	R	9.1 ± 0.62
Micrococcus aglis	7.9 ± 0.49	R	13.6 ± 0.64	9.1 ± 0.29	11.1 ± 0.62
Renibacterium salmoninarum	8.7 ± 0.20	11.8 ± 0.62	7.0 ± 0.65	11.0 ± 0.81	R
Marinococcus halophilus	6.0 ± 0.36	R	R	8.3 ± 0.47	8.9 ± 0.53
Planococcus citreus	R	6.5 ± 0.40	11.8 ± 0.62	R	R
Halomonas halodurans	6.5 ± 0.40	6.8 ± 0.62	6.4 ± 0.73	7.8 ± 0.23	7.2 ± 0.40
Micrococcus roseus	9.0 ± 0.40	R	R	8.1 ± 1.02	13.0 ± 0.81
Ancyclobacter aquaticus	10.7 ± 0.20	11.8 ± 0.62	14.1 ± 0.84	15.0 ± 0.81	R

R: Resistance.
Each value is the mean ± SD of three estimates.

DISCUSSION

Therefore it was the goal of this work to assess the ability of biofilm bacteria isolated from a tropical marine intertidal environment to produce antifouling compounds, identify the most active producing bacteria and to perform a preliminary characterization of the main compounds displaying antifouling activity. The symbiotic strains were isolated from the microalgal, *Caulerpa prolifera*. Totally five symbiotic bacterial strains belonging to four genera were isolated. They were: *Carnobacterium divergens*, *Alcaligens latus*, *Erythrobacter longus, Alcaligens faecalis and Azomonas agilis.* These symbiotic bacterial strains were mass cultured individually and extracted with ethyl acetate to screen for intracellular antifouling potentials.

This study showed that intertidal biofilm bacteria from tropical shores produce extracellular non-polar compounds with significant inhibiting activities. This in agreement with recent studies that bioactive metabolite production is more common among surface associated bacteria (both epibiotic and those derived from non-living substrata) compared to planktonic isolates, this phenomenon has been explained as ecological strategy exhibited by bacteria growing on the surfaces, those living in a highly competitive environment in which space and access to nutrients are limited, and may deter colonization of substrata by bacterial and crustacean competitors by producing antibiotics. (Burgess, *et al*., (1999), Hotmstorm, (1996).

REFERENCES

1. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Tyrok, M. 1966. Antibiotic susceptibility testing by standardized single disc method. *American J. Clin. Pathol.,* 45: 493-496.
2. Burgess, J.G., Jordan, M., Bregu, M.., Mearns Spragg, M., and Boyd, K.G.1999. Microbial antagonism: a neglected avenue and natural product research. *J. Biotechnol.,* 70: 27-32.
3. Clare A.S. 1996. Marine natural product antifoulants: status and potential, Biofouling., 9: 211-229.
4. Charackils, W.G., Flemming, H.C. and Geesey (eds), G.G. 1991. Biofouling: effects and control. Bio fouling and Bio corrosion in industrial water systems. *Springer-Verlag*, *Berlin,* 7-27.
5. Davey, M.E. and Toole, A.G. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev., 64: 847-867.
6. Haderlie, E.C. 1984. A brief overview of the effects of macrofouling, In: costlow J.D., Tipper R.C., (eds). Marine Bio deterioration an interdisciplinary study. *Noval Institute Press*, *Annapolis, Mary – land,* pp 163 -166.
7. Hotmstorm, C., James, S., Egan, S., Kjelleberg, S. 1996. Inhibition of common fouling organisms by pigmented marine bacterial isolates, Biofouling., 10: 251-259.
8. Sieburth, J.M. and Conover, J.T. 1965.Sargassumtannin, an antibiotic which retards fouling. Nature (Lond), 208: 52-53.
9. Rittschof, D. 2001. Natural products antifoulants and coating development. In: Mc Clintock J.B., Baker, B.J., (eds) *Marine Chemical Ecology, CRC, Florida,* pp 543-566.
10. Watnick, P.L., and Kotter, R. 1999. Steps in the development and *Vibrio cholera* for biofilm. *Mol*. *Microbiol*., 34: 586-595.