



Endobiotic bacteria in some seaweeds of Thondi coastal region in Palk Bay, Tamil Nadu, India

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Abstract

Fifteen bacterial isolates were enumerated from the six seaweeds collected along the coast of Thondi in Palk Bay, India. Among the 15 isolates, 6 bacteria (*Bacillus licheniformis*, *B. subtilis*, *B. pumilus*, *B. marinus*, *Staphylococcus aureus* and *Streptomyces coelicolar*) were Gram's +ve and 4 bacteria (*Pseudomonas fluorescens*, *Actinopolyspora* sp., *Vibrio mimicus* and *Serratia marscesens*) were Gram's -ve. The species of *Bacillus* were dominant among 10 bacteria isolated. *Bacillus subtilis* ASBs 05 and *Staphylococcus aureus* ASsa 06 were isolated from the red seaweed *Acanthophora spicifera* whereas from *Hypnea valentiae* 3 bacteria such as *Pseudomonas fluorescens* HVpf 03, *Actinopolyspora* sp. HVa 04 and *Bacillus licheniformis* HVbl 02 were isolated. The latter was also found in *Gracilaria edulis* GEbl 01 which was the only bacterium isolated from this alga. Of the 9 isolates of bacteria found in green seaweeds, *Bacillus subtilis* was isolated from *Enteromorpha intestinalis* EIbs 08 and *Ulva lactuca* ULbs 12 which was also found in red seaweed *Acanthophora spicifera* ASBs 05. Other two bacteria of 3 isolated from *E. intestinalis* were *Bacillus pumilus* EIbp 09 which was also found in *E. flexuosa* EFbp 10 whereas *Streptomyces coelicolar* EIsc 07 was exclusively found in *E. intestinalis*. Of the 2 bacteria isolated from *E. flexuosa*, *Vibrio mimicus* EFvm 11 was also found in *Ulva lactuca* ULvm 13. Among the 4 bacteria isolated from *Ulva lactuca*, the other two bacteria were *Serratia marscesens* ULsm 14 and *Bacillus marinus* ULbm 15 which were exclusively reported in this alga.

Keywords: Seaweeds, endobiotics, bacteria, Tamil Nadu, Palk Bay

Introduction

Bacterial diversity is expected to be high along the coastal waters due to the combined effects of euphotic zone and proximity of the shore which supplies sediments and particles from its various marine communities. The relationship between bacteria in the water column and their association with algae are still poorly known (Meusnier *et al.*, 2001). A typical 1ml of seawater contains 10^3 fungal cells, 10^6 bacteria and 10^7 viruses, including pathogens that cause widespread mortalities and microbes that initiate fouling on host surfaces (Kubanek *et al.*, 2003). Endosymbiotic *Pseudomonas* in *Caulerpa taxifolia* and six bacterial groups such as *Flavobacterium*, *Vibrio*, *Pseudomonas*, *Deleya*, *Escherichia* and Gram positive cocci in the tissues of green alga *Ulva pertusa* were identified (Chisholm

et al., 1996). Bacteria associated with seaweeds have the ability to produce chemical compounds which act against other marine bacteria and also against terrestrial pathogenic bacteria (Jensen and Fenical, 1994; Bernan *et al.*, 1997; Burgess *et al.*, 1999; Holmstrom and Kjelleberg, 1999). About 20% of bacterial strains isolated from marine algal surfaces are found to exhibit antibiotic production whereas from seawater it was only a few percent (Lemos *et al.*, 1986). The marine foliaceous green alga, *Ulva lactuca* loses its typical morphology when cultured aseptically in defined synthetic media and developed into a pincushion-like colony consisting of uniseriate branching filaments (Provasoli, 1958). The typical morphology was restored to a foliaceous form or tubular structure by the addition of appropriate marine bacteria to the medium (Provasoli and

Piritner,1980). Similar reports are made in *Ulva fasciata*, *Enteromorpha clathrata*, *E. linza* and *E.compressa* (Fries, 1975). These pincushion-like morphologies are also restored to typical fronds by addition of filter-sterilized supernatant medium from culture of specific marine bacteria (Tatewaki et al.,1993).

Studies on isolation of marine bacteria from seaweeds have been made elsewhere (Lafay et al., 1995; Nakanishi and Nishijima,1996;Imamura et al.,1997; Brock et al., 1998; Meusnier et al., 2001; Yan et al., 2003; Dhana Rao et al., 2005; Matsuo et al.,2005). But in India, works on bacterial flora of marine environment have been restricted to sediments and animals (Lokabharathi et al., 1990). So, in the present study an attempt was made to isolate the endobiotic bacteria associated with some seaweeds (marine macro-algae) occurring along the coast of Thondi in Palk Bay, Tamil Nadu, India.

Material and Methods

Collection of seaweeds

A portion of live, healthy and matured red seaweeds such as *Gracilaria edulis* (S.Gemelin) P.Silva, *Hypnea valentiae* (Turner) Montagne and *Acanthophora spicifera*(Vahl) Borgesen and green, *Enteromorpha intestinalis*, (Linnaeus) Nees, *E. flexuosa* (Wulfen) J. Agardh and *Ulva lactuca* Linnaeus collected along the coast of Thondi (Lat: 9° 44' 10" N and Long: 79° 00' 45" E, Palk Bay), Tamil Nadu, India on 15.05.2006 were transferred into separate clean plastic screw cap bottles containing 100ml sterilized seawater on the spot. The samples were brought to the laboratory immediately for further study. The external surface of algal thallus was scrapped gently using aseptic scalpel and washed thoroughly in sterile seawater at least thrice to remove extraneous particles and epiphytes under aseptic condition.

Isolation of endobiotic bacteria from seaweeds (Nakanishi and Nishijima,1996)

Seaweed weighing 1.0 g in fresh condition was homogenized with the help of sterilized glass mortar and pestle and the homogenate volume was

made up to 10 ml by using sterile seawater. The extract was kept for 30 min. for obtaining clear supernatant. Supernatant of alga was serially diluted with sterile seawater and spread on petriplates containing ca.15ml of zobel marine agar(sigma, USA)medium. The plates were kept for incubation at 27-30°C. The plates were observed every 24 hrs up to 5 days and bacterial colonies appeared were subsequently sub-cultured on fresh marine agar medium and single bacterial colonies were isolated.

Characterization of bacterial strains

The representative colonies were carefully picked, based on colony morphology including shape, size and colour. All the isolated bacterial strains were identified as per the procedure given in the Bergey's manual of systematic bacteriology. Biochemical parameters such as motility, Gram's staining, Indole test, Methyl red test, Voges Proskauer's test, Citrate utilization test, Lipase activity test, Gelatin liquefaction test, Oxidase and Catalase production were carried out. Fermentation of glucose, mannitol, xylose and lactose for the production of acid, alkali and gas by the isolates in the medium were studied (Brenner et al., 2005).

Results

Fifteen bacteria isolates designated as GEbl 01, HVbl 02, HVpf 03, HVa 04, ASbs 05, ASSa 06, Elsc 07, Elbs 08, Elbp 09, EFbp 10, EFvm 11, ULbs 12, ULvm 13, ULsm 14 and ULbm 15 were isolated from six seaweeds collected along the coast of Thondi in Palk Bay, Tamil Nadu, India. Morphology, Gram's staining and other tests (biochemical and sugar fermentations) described in the Bergey's Manual of systematic bacteriology (Brenner et al., 2005) were conducted on these isolates for identification and the results are presented in Tables 1 & 2. From the 15 bacterial isolates, 6 Gram's positive bacteria such as *Bacillus licheniformis*, *B. subtilis*, *B. pumilus*, *B. marinus*, *Staphylococcus aureus*, *Streptomyces coelicolar* and 4 Gram's negative bacteria such as *Pseudomonas fluorescens*, *Actinopolyspora* sp., *Vibrio mimicus* and *Serratia marscesens* were identified (Table 3).

Table 1. Morphological and biochemical characteristics of bacterial isolates from seaweeds found along the coast of Thondi, India during 2006

| IDENTIFICATION TEST | | Bacterial Isolates | | | | | | | | | |
|-----------------------|------------------------------|--------------------|-------------|-----------|--------------------|------------|------------|------------|------------|-------------|-------------|
| | | GEb 101 | ASbs 05 | | | | EIbP 09 | EFvm 11 | | | |
| | | HVb 1 02 | HVp f 03 | HVa 04 | EIbs 08 ULbs 12 | ASsa 06 | EIsc 07 | EFbp 10 | ULvm 13 | ULs m 14 | ULb m 15 |
| Morphological Test | Motility | + | + | - | + | + | + | + | + | + | + |
| | Shape & Arrangement | SR | SR | SR | SR& RC | C | SR | SR | SR | SR | SR |
| | Gram's Staining | + | - | - | + | + | + | + | - | - | + |
| | Indole Test | - | - | - | - | - | - | - | - | - | - |
| | Methyl red test | - | - | + | - | - | - | - | - | + | - |
| | Voges Proskauer Test | + | - | - | + | + | - | - | - | - | + |
| Biochemical Test | Citrate utilization Test | + | + | - | + | + | + | + | + | + | +/- |
| | Lipase activity test | - | - | + | + | + | - | - | - | - | - |
| | Starch hydrolysis test | - | + | - | ++ | + | + | - | ++ | + | + |
| | Proteolysis activity test | ++ | - | +/- | ++ | ++ | - | + | - | ++ | +/- |
| | Gelatin liquefaction test | - | - | - | - | - | - | - | - | - | - |
| | Oxidase test | + | +/- | - | + | + | + | + | + | + | + |
| | Catalase test | + | + | ++ | + | + | + | + | + | + | + |

SR - Short Rods, RC - Rods Chains, C - Cocci, + Positive, ++ Strong positive, +/- Weak positive

The species of *Bacillus* were dominant among 10 bacteria isolated. Only one strain of *Pseudomonas fluorescens* HVpf 03 was isolated from the red alga *Hypnea valentiae*. *Bacillus subtilis* ASbs 05 and *Staphylococcus aureus* ASsa 06 were isolated from the red alga *Acanthophora spicifera* whereas in *Hypnea valentiae* 3 bacteria such as *Pseudomonas fluorescens* HVpf 03, *Actinopolyspora* sp. HVa 04 and *Bacillus licheniformis* HVbl 02 were isolated. The latter was also associated in *Gracilaria edulis* (isolate GEbl 01) which was the only bacterium isolated from this alga. Of the 9 bacterial strains isolated from the green algae, *Bacillus subtilis* was isolated from the *Enteromorpha*

intestinalis (EIbs 08) and *Ulva lactuca* (ULbs 12) which was also found in red alga *Acanthophora spicifera* (ASbs 05). Other two bacteria isolated from *E.intestinalis* were *Bacillus pumilus* EIbp 09 which was also found in *E.flexuosa* (EFbp 10) and *Streptomyces coelicolar* EIsc 07 which was exclusively found in *E.intestinalis*. Of the 2 bacteria associated with *E. flexuosa*, *Vibrio mimicus* EFvm 11 was also found in *Ulva lactuca* (ULvm 13). Among the 4 bacteria isolated from *Ulva lactuca*, the other two bacteria were *Serratia marscesens* ULsm 14 and *Bacillus marinus* ULbm 15 which were exclusively reported in this alga (Table 3).

Table 2. Sugar fermentation test for bacteria isolates from seaweeds found along the coast of Thondi, India during 2006

| BACTERIAL ISOLATES | Duration (24 hrs) | | | | | | | | | | | | | | | |
|--------------------|-------------------|-----|----|----|----------|----|----|----|--------|----|----|----|---------|----|----|----|
| | Glucose | | | | Mannitol | | | | Xylose | | | | Lactose | | | |
| Culture conditions | Gr | Ac | Ga | Al | Gr | Ac | Ga | Al | Gr | Ac | Ga | Al | Gr | Ac | Ga | Al |
| GEbl 01 | | | | | | | | | | | | | | | | |
| HVbl 02 | + | + | - | - | + | - | + | + | + | - | - | + | + | + | - | - |
| HVpf 03 | + | + | - | - | + | + | - | - | + | + | - | - | + | ++ | - | - |
| Hva 04 | + | - | + | + | + | - | - | + | + | + | - | - | + | ++ | - | - |
| ASbs 05 | | | | | | | | | | | | | | | | |
| EIbs 08 | | | | | | | | | | | | | | | | |
| ULbs 12 | + | ++ | - | - | + | ++ | - | - | + | + | - | - | + | + | - | - |
| ASSa 06 | + | ++ | - | - | + | - | + | + | + | - | - | + | + | ++ | - | - |
| EIsc 07 | + | (+) | + | - | + | + | - | - | + | - | + | + | + | ++ | + | - |
| EIbp 09 | + | + | + | - | + | - | - | + | + | ++ | + | - | + | - | + | + |
| EFbp 10 | | | | | | | | | | | | | | | | |
| EFvm 11 | + | ++ | - | - | + | - | + | + | + | - | + | + | + | ++ | - | - |
| ULvm 13 | | | | | | | | | | | | | | | | |
| ULsm 14 | + | + | + | - | + | - | - | + | + | - | + | + | + | ++ | + | - |
| ULbm 15 | + | + | + | - | + | - | - | + | + | + | + | - | + | ++ | + | - |

+ Positive, - Negative, ++ Strong Positive, +/- Weak Positive
Gr. Growth, AC. Acid production, Ga. Gas production, Al. Alkaline

Discussion

Epibiotic and endobiotic bacteria associated with marine algae live in a highly competitive environment where space and access to nutrients are limited. These bacteria can produce secondary metabolites which inhibit the settlement of potential competitors, such as invertebrate larvae and can

antagonise other bacteria (Kubaneck *et al.*, 2003). On the other hand, substances produced by the bacteria when associated with seaweeds contribute for algal morphogenesis (Imamura *et al.*, 1997; Matsuo *et al.*, 2005).

Endosymbiotic bacteria *Pseudomonas* sp. was isolated from the coenocytic green alga *Caulerpa*

Table 3. Bacteria isolated from seaweeds found along the coast of Thondi, India during 2006

| Seaweeds | No. of Bacteria isolated | Bacteria |
|-------------------------------------|--------------------------|--|
| 1. <i>Gracilaria edulis</i> | 1 | <i>Bacillus licheniformis</i> GEbl 01 |
| 2. <i>Hypnea valentiae</i> | 3 | <i>Bacillus licheniformis</i> HVbl 02 |
| | | <i>Pseudomonas fluorescens</i> HVpf 03 |
| | | <i>Actinopolyspora</i> sp. HVa 04 |
| 3. <i>Acanthophora spicifera</i> | 2 | <i>Bacillus subtilis</i> ASbs 05 |
| | | <i>Staphylococcus aureus</i> ASSa 06 |
| 4. <i>Enteromorpha intestinalis</i> | 3 | <i>Streptomyces coelicolor</i> EIsc 07 |
| | | <i>Bacillus subtilis</i> EIbs 08 |
| | | <i>Bacillus pumilus</i> EIbp 09 |
| 5. <i>Enteromorpha flexuosa</i> | 2 | <i>Bacillus pumilus</i> EFbp 10 |
| | | <i>Vibrio mimicus</i> EFvm 11 |
| 6. <i>Ulva lactuca</i> | 4 | <i>Bacillus subtilis</i> ULbs 12 |
| | | <i>Vibrio mimicus</i> ULvm 13 |
| | | <i>Serratia marscesens</i> ULsm 14 |
| | | <i>Bacillus marinus</i> ULbm 15 |

taxifolia (Chisholm *et al.*, 1996). Six bacterial groups such as *Flavobacterium*, *Vibrio*, *Pseudomonas*, *Deleya*, *Escherichia* and Gram positive cocci were ubiquitous in the tissues of seaweeds (Matsuo *et al.*, 2005). In the present study, 4 bacteria such as *Bacillus subtilis*, *B. marinus*, *Vibrio mimicus* and *Serratia marscesens* were isolated from *Ulva lactuca* occurred in Palk Bay (India). Among 10 bacteria identified in this study, species of *Bacillus* (*Bacillus licheniformis*, *B. subtilis*, *B. pumilus*, *B. marinus*) were dominant. The bacterium *Bacillus licheniformis* isolated from the surface of brown alga, *Palmaria palmate* (Yan *et al.*, 2003) was also found in the red algae (*Gracilaria edulis* and *Hypnea valantiae*) in this study. The endosymbiotic bacteria, *Pseudomonas* isolated in green alga *Caulerpa taxifolia* (Chisholm *et al.*, 1996) was isolated from the red alga *Hypnea valantiae* (*Pseudomonas fluorescens*) collected at Thondi coast.

Bacillus subtilis and *Staphylococcus aureus* were isolated from the red seaweed *Acanthophora spicifera* occurred in this area whereas in *Hypnea valantiae* 3 bacteria such as *Pseudomonas fluorescens*, *Actinopolypora* sp. and *Bacillus licheniformis* were isolated. The latter was also associated in *Gracilaria edulis* which was the only bacterium isolated from this alga. Of the 9 bacterial strains isolated from the green seaweeds, *Bacillus subtilis* was associated with *Enteromorpha intestinalis* and *Ulva lactuca* which was also found in red seaweed *Acanthophora spicifera*. Other two bacteria isolated from *E.intestinalis* were *Bacillus pumilus* which was also found in *E.flexuos* whereas *Streptomyces coelicolar* was exclusively found in *E.intestinalis*. Of the 2 bacteria associated with *E. flexuosa*, *Vibrio mimicus* was also found in *Ulva lactuca*. Among the 4 bacteria isolated from *Ulva lactuca*, the other two bacteria were *Serratia marscesens* and *Bacillus marinus* which were exclusively reported in this alga.

Bacteria such as *Flavobacterium*, *Vibrio*, *Pseudomonas*, *Deleya*, *Escherichia* and Gram positive cocci were ubiquitous in the seaweeds such as *Ulva pertusa*, *Ulva conglobata*, *Enteromorpha intestinalis*, *Cladophora rugulosa*, *Chaetomorpha spiralis*, *Codium fragile*, *Colpomenia sinuosa*,

Scytosiphon lementaria, *Endarachne bingnamiae*, *Eisenia bicyelis*, *Undaria pinnatifida*, *Porphyra suborbiculata*, *Carpopeltis divaricata*, *Grateloupia lieida*, *Schizymenia dubyi*, *Plocamium telfainae*, *Hypnea Charoudes* and *Chondria dacyphylla* (Nakanishi and Nishijima, 1996). It is not known whether the seaweed associated bacteria are strictly host specific, or acquired from the local environment (Yan *et al.*, 2003). In the present study, presence of endobiotic bacterial strains of *Bacillus subtilis* in both the red (*Acanthopora spicifera*) and green seaweeds (*Enteromorpha intestinalis* and *Ulva lactuca*) suggested that bacterial association might be environmental specific, however seaweed associated bacteria are host specific would not be ruled out (Meusnier *et al.*, 2001).

In conclusion, presence of endobiotic bacteria was not uncommon among the seaweeds investigated. Large surface area may be the reason for harboring more number of bacteria in *Ulva lactuca* among the algae studied. Further study on seaweeds associated bacteria found along the Indian coastal waters for evaluating antibiotic potential may provide new bioactive compounds for compacting microbes with multi-drug resistance for global health problems. Besides, investigation of this relationship may reveal the extent and the nature of contribution of bacteria on seaweeds and *vice versa*.

Acknowledgements: The authors are grateful to The Principal, Alagappa Govt. Arts College and Prof. P. Chandrasekaran, Head of the Department of Botany, Alagappa Govt. Arts College, Karaikudi for the help and encouragements during the study.

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Received : 24/06/2009

Accepted : 16/07/2011

Published : 15/12/2011