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Short Communication: Marine organisms attached to seaweed surfaces in Jiaozhou Bay, China

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Scanning electron microscopy of the surfaces of the seaweeds *Laminaria japonica*, haploid *Porphyra yezoensis*, *Ulva pertusa* and the diploid conchocelis of *P. yezoensis* and *P. haitanensis* revealed *Vibrio* and *Micrococcus* to be abundant on the surfaces of *U. pertusa* and *P. yezoensis*. *Vibrio*, *Flavobacterium*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Corynebacterium* and other genera were isolated from the surfaces of *L. japonica*.

Key words: Attachment, identification, marine bacteria, seaweeds.

Bacterial attachment to marine flora and fauna is an important aspect of marine ecology (Colwell *et al.* 1980; Shiba & Taga 1980; Fletcher 1987) but the relationship between marine bacteria and seaweeds is not a simple phenomenon (Ambler *et al.* 1992). Although only a casual connection was thought to exist between seaweeds and marine bacteria, Provasoli & Printer (1980) reported that the morphology of *Ulva lactura* and *Monostroma oxyspermum* appeared abnormal under axenic culture but became normal when marine bacteria or yeasts were added to the culture medium. There seem to be factors in marine bacteria which influence the morphogenesis and development of seaweeds (Tatewaki *et al.* 1983). Here we report on a scanning electron microscope study of various seaweed surfaces and on the isolation of different microbial genera from them.

Materials and Methods

Algal Materials

The conchocelis (diploid stages) of *Porphyra yezoensis* and *P. hanitensis* were provided by the Experimental Marine Biology Laboratory, Chinese Academy of Science, and *Laminaria japonica*, diploid *Porphyra yezoensis* and *Ulva pertusa* were collected from the seashore of Jiaozhou Bay, Qingdao, China.

Scanning Electron Microscopy

The algae were cut into 1 × 1 cm pieces, washed with sea water sterilized by membrane filtration, fixed in 0.2 M sodium cacodylate buffer containing 2%, (w/v) glutaraldehyde, pH 7.2, for 24 h, rewashed twice in sodium cacodylate buffer, fixed in 1% OsO_4 for 2 h, washed twice with distilled water, dehydrated in a graded series of acetone, dried in vacuum and then coated with Au-Pd. The specimens were examined under a stereoscopic scanning electron microscope at 10 to 20 kV, and photographed (Sieburth & Thomas 1973).

Isolation and Identification of Attached Marine Bacteria

Algae were washed three times in sterile sea water and their surfaces then wiped with cotton swabs. The swabs were streaked on 2216E agar medium containing 0.1 g yeast extract, 0.5g peptone, 0.01 g $FePO_4$, 2% agar, 100 ml seawater (pH 7.0) and incubated at 25°C for 72 h. Selected colonies were sub-cultured separately on the same medium. After isolation, the marine bacteria were identified to genus level according to Bain & Shewan (1968). The identifying key characteristics included cell morphology, Gram reaction, flagellation and motility, colony pigmentation, gemma existence, luminescence, glucose catabolism, and oxidase and catalase reactions.

Results and Discussion

Electron Microscopy

Marine bacteria were observed distinctly attached to the surfaces of the seaweeds. The density of attached bacteria on haploid *P. yezoensis* and *U. pertusa*, was lower than that on *L. japonica* and higher than that on the diploid *Porphyra*. Diatoms were found attached in clusters to the surface of *Laminaria*. Spherical bacteria were abundant and formed a micro-fouling film on the middle part of *L. japonica* but this phenomenon was not as clearly observed in the other species. Sieburth & Tootle (1981) and Kogure *et al.* (1980) studied seasonal variation of marine bacteria and believed that survival of the marine microbial flora might be influenced by the geographical location, season, growth and

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Table 1. Organisms isolated from the seaweed surfaces.

Organism	No. of strains on:*				
	A	B	C	D	E
Vibrio sp.	2	11	7	—	—
Flavobacterium sp.	1	—	—	—	—
Pseudomonas sp.	1	—	—	—	—
Staphylococcus sp.	2	—	3	—	—
Bacillus sp.	1	—	—	—	—
Micrococcus sp.	—	2	5	—	—
Aeromonas sp.	—	1	—	—	—
Enterobacteriaceae	—	3	3	—	—
Corynebacterium sp.	1	—	1	—	—
Unidentified sp. 1	—	—	—	1	—
Unidentified sp. 2	1	—	—	—	—
Unidentified sp. 3	—	—	—	—	1
Total number	9	17	19	1	1

* A—*Laminaria japonica*; B—*Ulva pertusa*; C—diploid *Porphyra yezoensis*; D—Conchocelis of *Porphyra yezoensis*; E—Conchocelis of *Porphyra haitanensis*.

physiological state of the seaweeds. The microbial flora can apparently be beneficial to seaweeds.

Isolated Marine Bacteria

As shown in Table 1, the microbial flora attached to *Laminaria* were *Vibrio, Flavobacterium, Pseudomonas, Bacillus, Staphylococcus, Aeromonas* and *Corynebacterium* spp. There were also unidentified bacteria. Those organisms isolated from *Laminaria* were more diverse than those isolated from haploid *P. yezoensis* and *U. pertusa* and very few genera were isolated from *Porphyra* conchocelis stages, possibly because of their excretion of antibiotic substances. Most bacteria on *P. yezoensis* were found on the foliose thallus. *Vibrio* was the predominant genus on this seaweed, followed by *Staphylococcus, Micrococcus, Enterobacteriaceae* and *Corynebacteriaceae. Vibrio* was also more common than *Micrococcus, Aeromonas* or *Enterobacteriaceae* on *U. pertusa*. Overall, about 68% of the isolated strains were Gram-negative (Table 1), *Vibrio* and the *Enterobacteriaceae* predominating, followed by *Aeromonas, Staphylococcus* and unidentified genera. The Gram-positive isolates included *Micrococcus, Bacillus, Staphylococcus* and *Corynebacterium*, of which *Micrococcus* and *Staphylococcus* were the most common. Further study is necessary to understand the association between the microbial flora and seaweeds.

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