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Distribution of Aerobic Bacteria Which Contain Bacteriochlorophyll *a*

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Sixteen strains of aerobic bacteria which contain bacteriochlorophyll *a* were isolated from the samples collected in aerobic marine environments: thalli of *Enteromorpha linza*, *Porphyra* sp., *Sargussum horneri*; beach sand; and the surface seawater from Aburatsubo Inlet. When they occurred, their proportions among the aerobic heterotrophic populations ranged from 0.9 to 1.1% in the seaweed samples and from 1.2 to 6.3% in the beach sand samples and were 0.9% in the seawater sample. The results suggested that the aerobic photopigmented bacteria widely inhabit aerobic marine environments.

Bacteriochlorophyll *a* has been found only in photosynthetic bacteria. Most of the purple sulfur and nonsulfur bacteria contain this pigment as the major photopigment, whereas in the green sulfur bacteria it is a minor photopigment (3). Only the purple nonsulfur bacteria can grow aerobically, but the production of bacteriochlorophyll *a* in these bacteria is repressed at full atmospheric oxygen tension (2).

During the course of the investigations of heterotrophic bacteria attached to the high-tidal seaweed *Enteromorpha linza*, we isolated two different aerobic bacteria, OCh 101 (1) and OCh 114 (Shiba et al., Bull. Jpn. Soc. Sci. Fish, in press), which contain bacteriochlorophyll *a*. The strain OCh 101 is an orange-pigmented bacteria, and OCh 114 is pink pigmented. Both strains are gram-negative rods motile by means of polar or subpolar flagella, oxidase positive, and catalase positive, and both produce a small amount of acids from glucose, fructose, xylose, and the other carbohydrates. The amount of bacteriochlorophyll *a* in Och 114 was 5.5 nmol/mg (dry weight) and that in Och 101 was 0.9 nmol. These values are considerably higher than those reported for *Rhodopseudomonas sphaeroides* grown aerobically (2). Apart from the investigations of these bacteria from the physiological and phylogenetic points of view, their distribution in marine environments will be of considerable significance.

This report describes the result of a series of surveys of these bacteria in seaweeds, seawater, sand, and bottom sediment of Tokyo Bay and its adjacent areas.

MATERIALS AND METHODS

Collection of materials. The location of sampling stations is shown in Fig. 1. Beach sand, surface seawater, and the seaweeds *Monostroma nitidum*, *Enteromorpha linza*, *Ulva* sp., *Porphyra* sp., *Sargussum horneri*, and *Eisenia bicyclis* were collected at Aburatsubo Inlet in March and May 1978. Sand samples were collected at the tidal flat of Kisarazu Beach in September 1978. All of the samples collected were transferred to sterile glass bottles and immediately brought to the laboratory in an iced container. During the cruise of KT-78-13 aboard the research vessel *Tansei-maru*, Ocean Research Institute, University of Tokyo, Tokyo, Japan, seawater samples were collected at Tokyo Bay from various depths with an ORIT sampler (5). A bottom sediment sample was also collected during the cruise.

Enumeration of bacteria. The seaweed samples, which were cut with a sterile cork borer to give 1-cm^2 pieces, and the samples of sand and bottom sediment were blended for 3 min with sterile seawater. Tenfold dilutions of the samples were spread on an agar plate of PPES-II (5), which contains 0.2% Polypepton (Daigo Co.), 0.1% Soytone (Difco), 0.1% proteose-peptone no. 3 (Difco), 0.1% yeast extract (Difco), 0.01% ferric citrate, 1.5% agar (Difco), and 90.0% aged seawater. The pH was adjusted to 7.6. The plates were incubated at 20°C for 2 weeks before the colony count.

Examination of bacteriochlorophyll *a*. Colonies having characteristic orange (2.5YR 6-4/10-6, *Munsell Book of Color*, Munsell Co.) and pink (2.5-10R 6-7/8-10, *Munsell Book of Color*, Munsell Co.) color were isolated from the counting plates, and the isolates were grown in PPES-II liquid medium using a reciprocal shaker (Ishiyama Co.). The cells were harvested by centrifugation at 10,000 rpm for 10 min. The pigment was extracted from the cells with methanol, and the methanol solution was filtered in the dark. The filtrate was examined for light absorbancy with a spectrophotometer (Hitachi Co., model 124). The existence of bacteriochlorophyll *a* was ascertained by the fact that the filtrate has a peak in the region of 770 to 772 nm, which is characteristic of bacteriochlorophyll *a* (4).

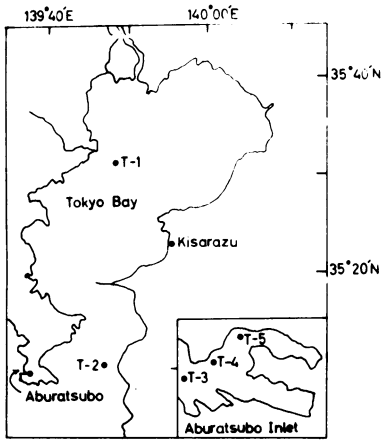

FIG. 1. *Location of sampling stations.*

Examination of anaerobic growth. The ability to grow anaerobically on PPES-II medium was tested by using a GasPak anaerobic system (Baltimore Biological Laboratory) with or without illumination. The light intensity was 500 to 1,000 lux.

RESULTS AND DISCUSSION

As the result of the examination of 8 seaweeds, 6 beach sand samples, 10 seawater samples, and 1 bottom sediment sample, 16 bacterial strains which contain bacteriochlorophyll *a* were isolated. As is shown in Table 1, nine strains were isolated from *E. linza*, *Porphyra* sp., and *S. horneri*. Six strains were isolated from three beach sand samples. One strain was isolated from the surface seawater of station T-4 of Aburatsubo Inlet. Other seawater samples, however, did not contain the photopigmented bacteria.

TABLE 1. *Distribution of aerobic bacteria which contain bacteriochlorophyll a*

Sample	Sampling day	Viable counts	Chromogenic bacteria* (%)	No. of chromogenic bacteria** tested	No. of photopigmented bacteria		Photopigmented bacteria (%)
					pink	orange	
Enteromorpha linza	March 25	431/plates***	30.6	46	2	2	0.9
Ulva sp.		3.2×10^6/cm^2	57.1	5	0	0	
Porphyra sp.		5.2×10^4	17.2	15	1	2	0.9
Enteromorpha linza	May 18	1.3×10^5	30.0	19	0	0	
Ulva sp.		1.0×10^6	75.0	32	0	0	
Monostroma nitidum		1.4×10^5	20.0	2	0	0	
Sargussum horneri		190/plates	17.2	13	2	0	1.1
Eisenia bicyclis		1.7×10^5/cm^2	0.8	4	0	0	
Sand(Aburatsubo)		64/plates	15.9	4	4		6.3
Sand(Kisarazu) I	Sept. 19	37	16.2	4	0	1	2.7
II		143	0.2	1	0	0	
III		116	4.3	5	0	0	
IV		158	1.9	2	0	0	
V		82	4.7	4	1	0	1.2
Seawater T-1 0 m	July 30	1.5×10^4/ml	0.0	0	0	0	
5		9.4×10^3	0.0	0	0	0	
10		6.9×10^4	7.9	5	0	0	
sed.*		93/plates	0.0	0	0	0	
T-2 0 m	July 30	1.0×10^3/ml	0.0	0	0	0	
5		3.6×10^3	2.8	0	0	0	
15		4.8×10^2	0.0	0	0	0	
25		1.8×10^3	0.0	0	0	0	
T-3 0	May 18	1.2×10^5	23.1	3	0	0	
T-4 0	May 18	1.1×10^5	12.0	4	1	0	0.9
T-5 0	May 18	1.4×10^5	9.0	2	0	0	

* Orange- and pink-pigmented bacteria.

** Bacteria having the color characteristic of aerobic photopigmented bacteria.

*** The numbers of bacterial colonies on agar plates are presented, when the surface area or the weight of samples were not determined. All of the percentage values are based on viable counts.

Also, we could not isolate these bacteria from bottom sediment. Of the 16 strains isolated, 11 strains were pink-pigmented bacteria, and 5 strains were orange-pigmented bacteria. None of them belongs to any known photosynthetic bacteria, because all of the strains were aerobic chemoorganotrophic bacteria and did not grow anaerobically in the light and the dark. As shown in Fig. 2, the methanol extract of the pink-pigmented bacteria has a relatively large peak in the region of 770 to 772 nm. The peak is higher than the other peak in the region of 460 to 480 nm. The extract of the orange-pigmented bacteria has a relatively small peak in the region of 770 to 772 nm.

The proportion of these photopigmented bacteria among the aerobic heterotrophic population ranged from 0.9 to 1.1% in the seaweed samples and from 1.2 to 6.3% in the sand samples and was 0.9% in the seawater sample. The viable bacterial counts of the samples were 10^4 to 10^5/cm^2 for the seaweed samples and 10^5/ml for the seawater sample. Thus, the population of the photopigmented bacteria could be estimated as 10^2 to 10^3/cm^2 and 10^3/ml, respectively. Although the bacterial numbers of the beach sand samples were not determined, they were generally 10^4 to 10^6/g according to past experiments (data not shown). Hence, the numbers of the photopigmented bacteria seem to be roughly 10^3/g of beach sand.

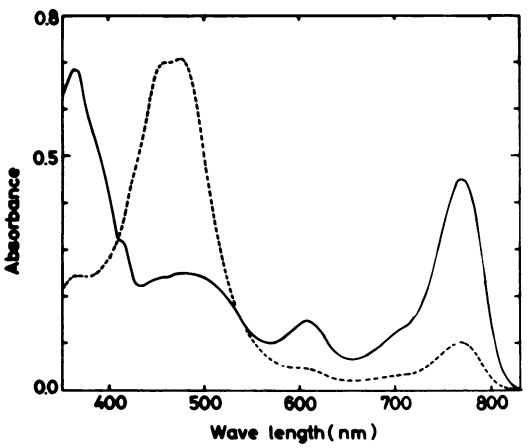

FIG. 2. *Absorption spectra of the methanol extracts of the pink-* (——) *and the orange-* (- - -) *photopigmented bacteria. The pink strain used is the isolate from the sand sample collected at Aburatsubo Inlet in May, and the orange strain is the isolate from E. linza collected at Aburatsubo Inlet in March. Approximately 0.5 g of the packed cells of the pink strain was extracted with 10 ml of methanol, and that of the orange strain was extracted with 50 ml of methanol. The methanol solutions were centrifuged at 10,000 rpm for 10 min.*

The large population density, together with the frequent isolations, suggests that the aerobic photopigmented bacteria widely inhabit seaweeds, beach sand, and seawater. Further experiments as to the physiological and morphological nature of the aerobic photopigmented bacteria are now in progress.

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LITERATURE CITED

1. **Harashima, K., T. Shiba, T. Totsuka, U. Simidu, and N. Taga.** 1978. Occurrence of bacteriochlorophyll *a* in a strain of an aerobic heterotrophic bacterium. Agric. Biol. Chem. **42**:1627-1628.
2. **Lascelles, J.** 1959. Adaptation to form bacteriochlorophyll in *Rhodopseudomonas sphaeroides*: changes in activity of enzymes concerned in pyrrole synthesis. Biochem. J. **72**:508-518.
3. **Pfennig, N., and H. G. Trüper.** 1974. Part I. The phototrophic bacteria, p. 24-75. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
4. **Smith, J. H., and A. Benitez.** 1955. Chlorophylls: analysis in plant materials, p. 142. *In* K. Paech and M. V. Tracey (ed.), Moderne Methode der Pflanzen-Analyse, 4th ed. Springer-Verlag, Berlin.
5. **Taga, N.** 1968. Some ecological aspects of marine bacteria in the Kuroshio current. Bull. Misaki Mar. Biol. Inst. Kyoto Univ. **12**:56-76.