Microbiological Studies of *Porphyra* plants—II. Bacteria Isolated from *Porphyra leucosticta* in Culture*

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Bacteria were isolated from the alga, *Porphyra leucosticta* THURET which had been grown healthy with a chemically defined medium in the laboratory by the methods of streaking on agar or the homogenizer in an effort of finding out what bacteria really accompanied.

Materials used were: the colour of the plant was normal dark brown, the plant cells observed microscopically were normal and the growth was the most prominent. The thalli were picked up from a culture flask of 500 ml of the artificial sea water and washed 2–3 times in a sterile sea water. This process was carried out in order to eliminate microorganisms attaching or adhering to the plant by chance, which might disturb results.

Classification was carried out through Bergey's Manual and gram-negative bacteria were specially identified according to the scheme proposed by SHEWAN *et al.*

Bacteria isolated were identified to the genus level as *Staphylococcus*, *Bacillus*, *Escherichia* etc., *Flavobacterium* and *Aeromonas*. The non-predatory relationship between *Porphyra leucosticta* THURET and *Staphylococcus* was suggested from the fact that this bacterium turned up in every time the isolation was carried out.

In a previous paper¹⁾ the methods of the isolation of bacteria from an alga, *Porphyra leucosticta* THURET, by using materials of different stages of the growth and of various appearances from healthy plants to abnormal ones that have cells with large vacuoles, which had been grown with an artificial sea water for five years through a monospore reproduction in the laboratory, were discussed.

In the present paper the writer has tried to find out what bacteria really accompanied the alga, *P. leucosticta*, which had been grown healthy with a chemically defined medium in the laboratory and reported 7 strains of isolated bacteria from the alga.

Material and Methods

Isolation. A special care was taken not to contaminate the plant with other microorganisms in the course of the isolation process. As a bacterial flora turned up in the method of streaking on agar differed in most case from that of the mortar or the homogenizer from the results previously reported,¹⁾ the both methods were used in this study. Materials used were: the colour of the plant was normal dark brown, the plant cells observed microscopically were normal and the growth was the most prominent. The culture has been incubated at 17°C to thalli of about 1 cm and thereafter 10°C, with the illumination by the fluorescent light of 3,000–5,000 lux intensity on a 8–16 light-dark

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cycle. The thalli were picked up from a culture flask of 500 ml of the artificial sea water and washed 2–3 times in a sterile sea water. This process was carried out in order to eliminate microorganisms attaching or adhering to the plant by chance, which might disturb results. An isolation medium was composed of the artificial sea water of culturing *P. leucosticta* with the constituents of ZoBell 2216 medium. The alga extract medium with the addition of 1.5% agar, which had been prepared by boiling 5 g of dried *P. leucosticta* in 80 ml of the artificial sea water, was also used. Bacteria isolated were incubated at 21–24°C.

Classification. Bacteria isolated were 7 strains. Classification was carried out through Bergey's Manual²⁾. Gram-negative bacteria were specially identified according to the scheme proposed by SHEWAN et al.³⁾.

Observations and tests employed for the classification are as follows:

1. Colonial morphology

Colony appearance was studied on streak plates containing well-separated colonies of each organism on agar plates of the isolation medium incubated at 21–24°C for 1 day to 1 week.

2. Gram stain

Gram stain was checked at least 3 times by the routine method.

3. Flagellation

Flagellation was investigated by the staining method of LÖFFLER. Each motile isolate was examined for flagella at least 3 times by staining.

4. Carbohydrate utilization

Bacteria were tested for their ability to dissimilate glucose in the HUGH and LEIFSON's medium made up by Nissui Seiyaku Co. Carbohydrate metabolism was also tested by using the marine oxidation-fermentation (MOF) medium of LEIFSON⁴⁾. To differentiate between fermentation and oxidation of carbohydrates, the writer followed the description appeared on the paper titled "Determination of carbohydrate metabolism of marine bacteria" by LEIFSON⁴⁾.

5. Oxidase test

Oxidase test⁵⁾ was applied to the colony on the streak plates of the isolation medium since this criterion is important for the determinative grouping of gram-negative bacteria according to SHEWAN⁶⁾. Cytochrome oxidase test paper simplified by Nissui Seiyaku Co. was used.

Results and Discussion

The results of tests for classification are shown in Tables 1–2 and from this, bacteria isolated were identified to the genus level as shown in Table 3.

The writer does not intend to classify the isolated bacteria but to know what kinds of bacteria really have an influence on the growth of the alga, *P. leucosticta*. Therefore the bacteria were studied and identified to the genus level as it serves his purpose.

Table 1. Morphological characteristics of marine organisms isolated from an alga, *P. leucosticta*.

Organisms	Gram stain	Flagellation	Form	Colonial appearance
Strain 1	Positive	_____	Spherical cells	Circular, white
Strain 2	Positive	Peritrichous	Rod shaped-cells	Circular, translucent, colourless
Strain 3	Positive	Peritrichous	Coccobacillary forms	Circular, translucent, white, become dim at margin
Strain 4	Negative	Peritrichous	Rod shaped-cells	Circular, brown yellow
Strain 5	Negative	Peritrichous	Filamentous forms	Circular, yellow
Strain 6	Negative	Polar monotrichous	Rod shaped-cells	Circular, translucent, white
Strain 7	Negative	Polar monotrichous	Rod shaped-cells	Circular, dirty white, become dim at margin

Table 2. Biochemical characteristics of marine organisms isolated from an alga, *P. leucosticta*

Organisms	Oxidase test	Carbohydrate utilization
Strain 1	Negative	_____
Strain 2	Negative	_____
Strain 3	Negative	_____
Strain 4	Positive	_____
Strain 5	Negative	_____
Strain 6	Positive	Fermentative, much gas
Strain 7	Positive	Fermentative, much gas

Table 3. Identified genera from each strain.

Organisms	Genus name
Strain 1	<i>Staphylococcus</i>
Strain 2	<i>Bacillus</i>
Strain 3	<i>Escherichia</i> etc.
Strain 4	<i>Flavobacterium</i>
Strain 5	<i>Flavobacterium</i>
Strain 6	<i>Aeromonas</i>
Strain 7	<i>Aeromonas</i>

CHAN and MACMANUS⁷⁾ isolated several bacteria belonging to the genera *Pseudomonas*, *Vibrio*, *Flavobacterium*, *Achromobacter* or *Alkaligenes*, *Escherichia* etc., *Staphylococcus*, *Sarcina* and a pink yeast *Rhodotorula*? from the algae, *Polysiphonia* and *Ascophyllum*, and the environmental sea water. However it is not known whether the algae had been washed or not for eliminating microorganisms attaching or adhering to the plant by chance before the isolation of bacteria was done.

MURCHELANO and BROWN⁸⁾ described that cultures of the algae, *Monochrysis lutheri*, *Isochrysis galbana*, *Phaeodactylum tricormutum*, *Dicrateria* sp., *Chlorella* sp. and *Tetraselmis maculata* contained bacteria belonging to the genera *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Vibrio*, *Bacillus* and *Corynebacterium*. BERLAND et al.⁹⁾ found that cultures of the algae, *Asterionella japonica*, *Chaetoceros lauderi*, *Lauderia borealis*, *Leptocylindricus danicus*, *Phaeodactylum tricormutum* and *Stichochrysis immobilis* contained bacteria belonging to the genera *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Vibrio*,

Agarbacterium, *Xanthomonas*, *Micrococcus* and *Staphylococcus*. As both instances are the isolation of bacteria from the cultures of algae and not the isolation of bacteria which attach or adhere to or penetrate the algae, bacteria isolated are not necessarily to affect the growth of *P. leucosticta*.

The writer has worked on isolation many times and bacteria isolated so far were *Staphylococcus*, *Bacillus*, *Escherichia* etc., *Flavobacterium* and *Aeromonas*. *Flavobacterium* was always appeared in large quantity in his study as well as in their results reported by some workers mentioned above. Further studies are needed on the occurrence of *Flavobacterium* if it has a significant meaning on the growth of *P. leucosticta*. *Staphylococcus* always appeared when thalli with micrococci were used in the isolation of bacteria. It is thought that the micrococci might be *Staphylococcus* and it might have some sorts of relation with *P. leucosticta*. Continuing investigation on both the physiology of the bacterium in question and the effect of it on the growth of *P. leucosticta* in bacteria-free culture are necessary components of truly critical studies of non-predatory relationships.

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