Microbiological Studies of *Porphyra* plants-I.

Studies of Bacteria Isolation Methods

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Introduction

Unialgal cultures may serve to investigate the effects of physical or chemical factors and of inorganic salts. However bacteria-free cultures must be employed for detecting the effects of the orangic substances as these substances are excreted or secreted in the natural environment by some microorganisms[1].

Many workers [1) 2) 3) 4) 5) 6) 7) 8)] have succeeded in growing the algae of a simple form like unialgal or uniseriate in bacteria-free culture. The algae purified have been known to grow normally and it was elucidated that some vitamins definitely had worked on these algae as growth factors. In spite of these excellent investigations, the axenic culture of thalloid algae and polysiphonous ones have not been reported yet.

The writer has long been working on the nutrition of *Porphyra* plants in bacteria-free condition. Vitamins, plant hormons, amino acids and many other substances have been examined if they have any special effect the plants to be healthy and natural. The plants, however, free from bacteria seldom grow with these substances, though they remain alive quite a long time. He, therefore, came to conclusion that the *Porphyra* plants do need unknown metabolites of bacteria either as nutrients or as the stabilizer of microenvironment on or very near the surface of the plants in order to keep the normal assimilation of nutrients.

As it was apparent from the facts described above that bacteria associated did some work on the growth of *Porphyra* plants, he took another way to make clear how bacteria would work on them. The following steps to work on it was thought: the isolation of bacteria and the investigation of their physiology would come first to do, next to do, if suspected bacteria are found, is to transfer them into the bactera-free culture of *Porphyra* plants and see what to do, and finally the association between *Porphyra* plants and bacteria would come to study further.

The present paper describes the methods of the isolation of bacteria from the alga, *Porphyra leucosticta* THURET.

Isolation Methods

Materials used in this experiment were *Porphyra leucosticta* THURET of different stages of the growth and of various appearances from healthy plants to abnornal ones having

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large vacuoles, which had grown in the laboratory with an artificial sea water for five years through a monospore reproduction at 17°C to thalli of about 1 cm and thereafter 10°C, with the illumination by fluorescent light of 3000—5000 lux intensity on a 8—16 light-dark cycle. The plant about several centimeter long after two months of sporulation was served for the isolation of bacteria. It was washed out other microorganisms attaching to the plants by chance 2—3 times in a sterilized sea water before doing the isolation procedure for getting repeating results. It is thought that the microorganisms merely attaching are removed in washing process but microorganisms which exist in the mucilage layer are not likely to be taken out.

The medium employed was the artificial sea water of *Porphyra* plants with addition of the constituents of ZoBell 2216. The bacteria were incubated at 21—24°C. The isolation procedure was carried out in specially prepared room and all tools used was sterilized in prior to.

In present paper 8 strains were isolated, being studied the isolation methods described below. These strains of which morphology is shown in Table I are tentatively called strain 1—8.

Table I

Summary of morphological characteristics of bacteria isolated from an alga, P. *leucosticta*

Organisms	Motility	Form	Colonial appearance
strain 1	−	Spherical cells	Circular, white
strain 2	+	Rod shaped-cells	Circular, translucent, colourless
strain 3	+	Coccobacillary form cells	Circular, translucent, white, become dim at margin
strain 4	+	Rod shaped-cells	Circular, brown yellow
strain 5	+	Filamentous form	Circular, yellow
strain 6	+	Rod shaped-cells	Circular, translucent, white
strain 7	+	Rod shaped-cells	Circular, dirty white, become dim at margin
strain 8	+	Rod shaped-cells	Circular, yellow white

* + motile, − non motile

1. Streaking on agar

Material plants were cut into about 5 mm square, put them on the agar plate of the isolation medium and drugged on it with a capillary pipet or a platinum rod. This method has been tried 5 times and results are shown in Table II.

2. Using motar

Material plants of about 5 cm long and 2 cm wide at maximum were put into the

Table II

Bacteria isolated by the method of streaking on agar

Experiment number / Organisms	1	2	3	4	5
strain 1					
strain 2					
strain 3				○	
strain 4			○		
strain 5		○			○
strain 6	○	○	○	○	○
strain 7	○		○		○
strain 8			○		○

* ○ indicates strain appeared.

Table III

Bacteria isolated by the method of motar

Experiment number / Organisms	1	2	3
strain 1	○		○
strain 2	○	○	○
strain 3	○		○
strain 4		○	
strain 5		○	
strain 6			○
strain 7	○	○	
strain 8	○		

* ○ indicates strain appeared.

Table IV

Bacteria isolated by the method of homogenizer

Experiment number / Organisms	1	2	3	4
strain 1		○	○	○
strain 2	○	○	○	○
strain 3	○	○		
strain 4	○			
strain 5	○		○	
strain 6			○	○
strain 7		○		
strain 8	○	○		○

* ○ indicates strain appeared.

motar with 1 ml of the sterilized artificial sea water and crushed into fine pieces. 0.05 ml of it was brought into the Petri dish with the isolation medium of the temperature 40—45°C and then stirred. This method has been tried 3 times and results are shown in Table III.

3. Using homogenizer

Material plants of the same size mentioned above item 2, using motar, were put into the cup of the homogenizer together with 10 ml of a sterilized artificial sea water and set to work at 12000 rpm. for 1—2 minutes. 0.1—0.2 ml of it was brought into the Petri dish with the isolation medium of the temperature of 40—45°C and then stirred. This method has been tried 4 times and results are shown in Table IV

Discussion

Besides the methods described above, the writer practised an isolation method of putting thalli on the agar plate of the medium only to get the same result as that of streaking on agar.

The bacterial floras observed by the method of streaking on agar differed in most instances from those by the methods of homogenizer or motar. It might be due that

the effect of dilution was produced with the methods of homogenizer or motar as a sterile artificial sea water had been added in the process, and in the method of streaking on agar, only bacteria existing on the surface of the plants were able to be isolated while with using homogenizer or motar, bacteria penetrating into the cells as well as ones attaching the surface of the plants could be isolated.

Bacteria shown up in the method of streaking on agar were also isolated with the methods of homogenizer or motar. Of many experiments, however, bacteria isolated in the method of streaking on agar sometimes did not show up in those of homogenizer or motar. It is not natural to take it as the bacteria shown up in the method of streaking on agar did not exist on the plant used as materials of the methods of homogenizer or motar but natural to take it as it is attributed to the methods employed.

For these reasons mentioned above, the good result should have been obtained by the methods of homogenizer or motar together with the method of streaking on agar.

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Summary

1. Various isolation methods were studied in the effort of finding out a suitable method of the isolation of bacteria from the alga, *Porphyra leucosticta* THURET.
2. It was described that the good result should have been obtained by the method of using homogenizer or motar together with the method of steaking on agar.

References

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正　　誤　　表 (Errata)

頁 Page	行・図・表 Line·Figure·Table	誤 Error	正 Correction
1	上から2	膀胱内異物成形	膀胱内異物形成
1	上から5	MARING	MARINE
2	下から12	20間	20日間
3	Table 1—1	Numbers of larvae was seated	Numbers of larvae seated
3	Table 1—2	Numbers of larvae was seated	Numbers of larvae seated
4	Fig. 2.	(mm/a day)	(mm/day)
5	Fig. 4.	ACCUMLATIVE	ACCUMULATIVE
6	Fig. 5.	FOREING	FOREIGN
6	Fig. 5.	○ : A—series	○ : B—series
6	Fig. 6.	DODY LENGTH	BODY LENGTH
8	上から3	塩分濃度	塩分
8	上から13	カルシュームは	カルシュームの
9	上から3	塩分濃度	塩分
12	上から11	ブァン氏液	ブアン氏液
12	上から18	フ化後の	孵化後の
12	上から18	フ化直後	孵化直後
13	上から14	(Figs. 1～2)	(Fig. 1.)
13	Fig. 1.	Foreing	Foreign
14	Table 1	Table. 1	Table 1
14	Table 1	neutral	Neutral
14	Table 1	absolute	Absolute
15	上から4	合体していまい	合体してしまい
19	上から16	special effect the plants	special effect to the plants
19	脚注2	Contrịbntion	Contribution