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Microbiological Studies of *Porphyra* Plants-V.

On the Relation between Bacteria and *Porphyra* diseases

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It has been reported that cells of *Porphyra* thalli turn pale in colour and die when cultured in a medium dominated by a certain bacterium.[1] Moreover, white and dead cells were found to increase in number when *Vibrio* appeared predominantly on cultivated *Porphyra*.[2] In order to elucidate the above results, further studies were conducted on the relation among the increase in number of white and dead cells, the predominance of certain bacteria and the appearance of *Vibrio* in cultivated and uncultivated *Porphyra* areas.

Materials and methods

Observations on the growth and bacterial flora of *Porphyra* thalli were made in cultivated *Porphyra* areas in fiscal* 1975 and in uncultivated *Porphyra* areas in fiscal 1973 and 1974 located in front of the laboratory. Collected materials were quickly brought back to the laboratory and were observed with the naked eyes and under a microscope. Bacteria were also isolated from the *Porphyra* thalli.

1. Isolation

Bacteria attached to *Porphyra* thalli were isolated by one or both of the methods described in a previous paper,[3] i. e., streaking on agar and using a homogenizer.

2. Medium

A modified ZoBell 2216 E medium (Table 1) was used for isolating and preserving bacteria.

3. Identification

Isolated bacteria were identified to the generic level with BERGEY' manual[4] and by gram negative rods mainly with SHEWAN'S scheme.[5]

Results and discussion

The general appearance of the thalli and the data on the attached bacteria are presented in Tables 2~4. In fiscal 1973, strain 48-5 formed 0.4 % of the bacterial flora on the thalli collected on Jan. 17, 1974. White and dead cells were abundantly observed on the thalli collected on that day, but few such cells were found on the other days. In fiscal 1974, strain 49-5 formed 22.1 %

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* The fiscal year of *Porphyra* culture is October to May in Japan.

of the bacterial flora on the thalli collected Jan. 20, 1975. White and dead cells were abundantly observed on the thalli 3 days after strain 49-5 appeared, but were rarely found in the absence of strain 49-5. In fiscal 1975, strain 50-5 formed 20 % and 30.7 % of the bacterial flora on the thalli collected on Nov. 6 and 13, 1975, respectively. White and dead cells were abundantly observed on the thalli collected on Nov. 10, 13 and 15, but were not found on the other days. In other words, a lot of white and dead cells were found on the days when strain 48-5 appeared in fiscal 1973, 3 days after strain 49-5 appeared in fiscal 1974 and 2 and 4 days after strain 50-5 appeared in fiscal 1975.

Strains 48-5, 49-5, and 50-5 were all identified as *Beneckea*. Morphological and biochemical characteristics of strain 48-5 are given in Table 5.

FUJITA et al.[6] reported that the red spots occurred *in vitro* on *Porphyra* thalli after inoculation of *Beneckea*. These spots eventually spread to surface of the host. Therefore, they thought that this bacterium had some role in causing a *Porphyra* disease described by SUTO et al.[7]

According to KATADA,[8] *Porphyra* utilizes large amounts of nutrients in the cultivated areas when the seawater temperature drops gradually at the beginn-

Table 1. Composition of the medium used for bacteria isolation and preservation

Chemically defined medium	100	mℓ
Meat extract	0.2	g
Peptone	0.5	g
Potassium nitrate	0.05	g
Agar	1.5	g
Chemically defined medium 100 mℓ=		
NaCl	2.4	g
$MgSO_4$ $7H_2O$	0.8	g
KCl	70	mg
$CaCl_2$ $2H_2O$	55	mg
$NaHCO_3$	16.8	mg
$NaNO_3$	20	mg
Na_2 glyceroPO_4	2.5	mg
Metal solution	1	mℓ
Metal solution 1 mℓ=		
H_3BO_3	0.2	mg
$FeCl_3$ $6H_2O$	0.048	mg
$MnCl_2$ $4H_2O$	0.144	mg
$CoCl_2$ $6H_2O$	0.04	mg
$ZnCl_2$	0.01	mg
Na_2EDTA	1	mg

Table 2. Observations on the cells and bacterial flora of *Porphyra* collected from rocks in fiscal 1973.

Date	Number of bacteria	Bacterial flora		Remarks of *Porphyra* cells
Dec. 4	2×10^4	48 — 1	3.8%	Healthy
		48 — 2	4.6%	
		48 — 3	6.1%	
		48 — 4	75.7%	
		48 — 5	0 %	
		Others	9.8%	
Dec. 11	6×10^3	48 — 1	11.8%	Healthy
		48 — 2	41.7%	
		48 — 3	0 %	
		48 — 4	27.8%	
		48 — 5	0 %	
		Others	19.4%	
Dec. 18	4×10^4	48 — 1	4.3%	Healthy
		48 — 2	43.1%	
		48 — 3	8.6%	
		48 — 4	43.1%	
		48 — 5	0 %	
		Others	0.9%	
Dec. 21	2×10^4	48 — 1	8.2%	Healthy
		48 — 2	8.2%	
		48 — 3	1.6%	
		48 — 4	82.6%	
		48 — 5	0 %	
		Others	0 %	
Dec. 25	4×10^4	48 — 1	12.1%	Healthy
		48 — 2	18.1%	
		48 — 3	9.6%	
		48 — 4	60.2%	
		48 — 5	0 %	
		Others	0 %	
Dec. 27	1×10^4	48 — 1	17.9%	Healthy
		48 — 2	0 %	
		48 — 3	0 %	
		48 — 4	71.4%	
		48 — 5	0 %	
		Others	10.7%	
Jan. 5	8×10^3	48 — 1	24.3%	Faded chromatophores
		48 — 2	5.4%	
		48 — 3	2.7%	Groups of clumped cells
		48 — 4	67.6%	
		48 — 5	0 %	
		Others	0 %	
Jan. 9	8×10^3	48 — 1	7.7%	Faded chromatophores
		48 — 2	7.7%	
		48 — 3	7.7%	
		48 — 4	69.2%	
		48 — 5	0 %	
		Others	7.7 %	
Jan. 17	1×10^5	48 — 1	0.9%	Faded chromatophores
		48 — 2	34.5%	
		48 — 3	21.6%	White and dead cells
		48 — 4	42.6%	
		48 — 5	0.4%	
		Others	0 %	
Jan. 22	1×10^4	48 — 1	0 %	Faded chromatophores
		48 — 2	14.8%	
		48 — 3	24.6%	
		48 — 4	55.7%	
		48 — 5	0 %	
		Others	14.9%	

Table 3. Observations on the cells and bacterial flora of *Porphyra* collected from rocks in fiscal 1974.

Date	Number of bacteria	Bacterial flora	Remarks of *Porphyra* cells
Nov. 22	2×10^4	49 — 1 100 % 49 — 2 0 % 49 — 3 0 % 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Nov. 28	2×10^4	49 — 1 1.8% 49 — 2 8.9% 49 — 3 89.3% 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Dec. 2	2×10^2	49 — 1 0 % 49 — 2 0 % 49 — 3 100 % 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Dec. 5	2×10^3	49 — 1 42.3% 49 — 2 0 % 49 — 3 42.3% 49 — 4 0 % 49 — 5 0 % Others 15.4%	Healthy
Dec. 11	2×10^4	49 — 1 33.6% 49 — 2 29.7% 49 — 3 36.7% 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Dec. 19	2×10^2	49 — 1 0 % 49 — 2 0 % 49 — 3 100 % 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Dec. 23	2×10^2	49 — 1 0 % 49 — 2 0 % 49 — 3 100 % 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Dec. 27	8×10^2	49 — 1 25 % 49 — 2 0 % 49 — 3 75 % 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy
Jan. 6	2×10^4	49 — 1 2.8% 49 — 2 21.7% 49 — 3 75.5% 49 — 4 0 % 49 — 5 0 % Others 0 %	Healthy

Table 3. Continued

Date	Number of bacteria	Bacterial flora		Remarks of *Porphyra* cells
Jan. 13	2×10^3	49 — 1	20.4%	Healthy
		49 — 2	0 %	
		49 — 3	29.6%	
		49 — 4	20.4%	
		49 — 5	0 %	
		Others	0 %	
Jan. 18	4×10^4	49 — 1	0 %	Enlarged vacuole
		49 — 2	49.2%	
		49 — 3	50.8%	
		49 — 4	0 %	
		49 — 5	0 %	
		Others	0 %	
Jan. 20	1×10^4	49 — 1	5.9 %	Healthy
		49 — 2	2.9 %	
		49 — 3	11.3 %	
		49 — 4	51.9 %	
		49 — 5	22.1 %	
		Others	5.9 %	
Jan. 23	2×10^4	49 — 1	0 %	White and dead cells
		49 — 2	0 %	
		49 — 3	0 %	
		49 — 4	100 %	
		49 — 5	0 %	
		Others	0 %	
Jan. 28	6×10^3	49 — 1	0 %	Faded chromatophores
		49 — 2	3.7%	
		49 — 3	0 %	
		49 — 4	96.3%	
		49 — 5	0 %	
		Others	0 %	
Jan. 31	2×10^3	49 — 1	10.6%	Healthy
		49 — 2	0 %	
		49 — 3	33.6%	
		49 — 4	33.6%	
		49 — 5	0 %	
		Others	22.2%	

Table 4. Observations on the cells and bacterial flora of *Porphyra* collected from nets in fiscal 1975.

Date	Number of bacteria	Bacterial flora	Remarks of *Porphyra* cells
Oct. 30	8×10^3	50 — 1 45 % 50 — 2 17.5% 50 — 3 2.5% 50 — 4 32.5% 50 — 5 0 % Others 2.5%	Healthy
Nov. 3	4×10^3	50 — 1 22.7% 50 — 2 18.2% 50 — 3 0 % 50 — 4 59.1% 50 — 5 0 % Others 0 %	Healthy
Nov. 6	4×10^3	50 — 1 25 % 50 — 2 10 % 50 — 3 5 % 50 — 4 40 % 50 — 5 20 % Others 0 %	Healthy
Nov. 10	8×10^3	50 — 1 56.1% 50 — 2 12.2% 50 — 3 2.4% 50 — 4 29.3% 50 — 5 0 % Others 0 %	White and dead cells
Nov. 13	1×10^4	50 — 1 10.2% 50 — 2 16.3% 50 — 3 20.4% 50 — 4 22.4% 50 — 5 30.7% Others 0 %	White and dead cells
Nov. 15	2×10^5	50 — 1 50 % 50 — 2 25 % 50 — 3 25 % 50 — 4 0 % 50 — 5 0 % Others 0 %	White and dead cells
Nov. 18	4×10^4	50 — 1 2.8% 50 — 2 28.6% 50 — 3 40.0% 50 — 4 28.6% 50 — 5 0 % Others 0 %	Healthy
Nov. 22	8×10^5	50 — 1 0 % 50 — 2 27.8% 50 — 3 33.3% 50 — 4 38.9% 50 — 5 0 % Others 0 %	Healthy
Nov. 25	4×10^5	50 — 1 8.3% 50 — 2 0 % 50 — 3 8.3% 50 — 4 83.4% 50 — 5 0 % Others 0 %	Healthy

Table 5. Morphological and biochemical characteristics of strain 48-5.

Characteristics	Isolated bacteria
Cells form	Moderate straight round
Gram stain	−
Motility	+
Flagella	Petrichous
Pigmentation	−
Kovacs oxidase	+
Catalase	+
Acid by (MOF) from	
Glucose	F
Maltose	+
Mannitol	+
Ribose	+
Sorbitol	+
Sucrose	+
Lactose	+
Arabinose	+
Fructose	+
Galactose	+
Xylose	+
Raffinose	+
Trehalose	+
Mannose	+
VP test	−
MR test	+
Indol	+
Hydrolysis	
Starch	+
Chitin	+
Gelatin	+
Casein	+
Tween 80	+
Alginate	−
Cellulose	−
Agar	−
Gelatin liquefaction	+
NO_3 reduction	+
NO_2 reduction	+
TMAO	−
Litmus milk	Peptonization
Decarboxylation	
Lysine	−
Ornithine	−
Arginine	−
Sensitivity to 0/129	±
H_2S production	+
Phosphatase	+
Growth at 10% NaCl	+
Sea water requirement	H
Growth at	
0 °C	−
5 °C	−
30 °C	+
37 °C	+
40 °C	+
Gluconate oxidation	−
NH_3 from Peptone	+
Citrate	+
GC moles %	44.2

Table 6. Maximum difference between atmospheric and seawater temperature during the period of exposure of *Porphyra* to air greater than 4 hours per day

In fiscal 1973

Date	Temperature difference
Jan. 7, 1974	9.1°C
Jan. 12, 1974	9.8°C
Jan. 19, 1974	9.0°C
Jan. 20, 1974	7.9°C
Jan. 27, 1974	10.1°C

In fiscal 1974

Date	Temperature difference
Jan. 12, 1975	8.9°C
Jan. 13, 1975	9.5°C
Jan. 28, 1975	4.0°C
Jan. 29, 1975	9.0°C
Jan. 31, 1975	9.3°C

In fiscal 1975

Date	Temperature difference
Nov. 4, 1975	8.4°C
Nov. 22, 1975	9.2°C
Nov. 23, 1975	9.4°C

ing of the harvesting season. He speculated that dissolved nutrients, such as nitrogen, phosphorus and carbonic acid substances become insufficient for the normal metabolism, and consequently, *Porphyra* was infected with "white wasting disease", "white blight", or "Shirogusare-sho".

Fishermen say that *Porphyra* is apt to be infected with disease when the difference between the atmospheric and seawater temperatures is big at dawn ebb tide. FUJIKAWA.[9] have found that 2-4 hours of exposure per day is optimal for *Porphyra* growth.

In order to understand the relationship of environmental factors to *Porphyra* diseases, desiccation and temperatures in cultivated areas were examined. At first, the days when plants exposed to the atmosphere for over 4 hours at dawn and when white and dead cells were abundantly observed on the thalli

were enumerated. The difference in the atmospheric and seawater temperatures on these days is shown in Table 6. From the data shown in Tables 2, 3, 4, and 6, in fiscal 1973, a temperature difference of 9.8°C occurred 5 days before the appearance of *Beneckea*; in fiscal 1974, a temperature difference of 9.5°C occurred 7 days before the appearance of *Beneckea*; and in fiscal 1975, a temperature difference of 8.4°C occurred 2, and 9 days before the appearance of *Beneckea*. In other words, *Beneckea* appeared on the 2 nd to 9 th day after the temperature difference was greater than 8.0°C.

On the other hand, in fiscal 1975, unhealthy thalli with white and dead cells or with cells containing granules were found in cultivated *Porphyra* on Nov. 10. These damaged cells were found to increase remarkably in number with time and many *Porphyra* thalli were soon lost from the cultivation nets. From the above facts, it is apparent that soon after the appearance of *Beneckea*, the number of white and cells of *Porphyra* increases.

Generally speaking, the chromatophores transformed first and then the colour of the cells faded. In course of time, the cells shrunk in size and cell contents became homogenious. The cell colour was red at this stage but later turned white. White and dead cells increased in number irrespective of thallus size. Abnormal cells containing granules were observed at times. This symptom is characteristic of "white wasting disease", "white spot", or "Gijishirogusare-sho" described by SUTO et al.[7] Therefore, *Beneckea* may be one of pathogenes which causes this disease.

As reported in a previous paper,[2] it appeared that the predominance of *Vibrio* might have some role in influencing the occurrence of wtite and dead cells but a relation between this pathogen and the process leading to the appearance of white and dead cells observed in this study was not found.

In summary, *Beneckea* appeared on the 2 nd to 9 th day after *Porphyra* was exposed to the atmosphere for more than 4 hours and, at the same time, the difference in temperature exceeded 8°C; then the number of white and dead cells increased markedly and plants died 4 days after the appearance of *Beneckea*.

In conclusion, it is speculated that the disease is caused by *Beneckea* which prevails on *Porphyra* thalli under certain natural conditions. However, there were a few cases in the past three fiscal years where *Benechea* populations were not found on the thalli even under the appropriate environmental conditions. It is not certain that *Beneckea* requires the repetition of the above mentioned conditions or only that the environmental factors must include these.

Summary

White and dead cells were found to increase in number when *Vibrio* appeared predominantly on cultivated *Porphyra*. In order to elucidate the above results, further studies were conducted on the relation among the increase in number of white and dead cells, the predominance of certain bacteria and the appearance of *Vibrio* in cultivated and uncultivated *Porphyra* areas. A lot of white and dead cells were found on the days when strain 48-5 appeared in fiscal 1973, 3 days after strain 49-5 appeared in fiscal 1974, and 2 and 4 days after strain 50-5 appeared in fiscal 1975. These strains were all identified as *Beneckea*. To understand the relationship of environmental factors to *Porphyra* diseases, desiccation and temperatures in cultivated areas were examined. The days when plants exposed to the atmosphere for over 4 hours at dawn and when white and dead cells were abundantly observed on the thalli were enumerated. A temperature difference of 9.8°C occurred 5 days before the appearance of *Beneckea* in fiscal 1973, 9.5°C occurred 7 days before the appearance of *Beneckea* in fiscal 1974, and 8.4°C occurred 2, and 9 days before the appearance of *Beneckea* in fiscal 1975. In summary, *Beneckea* appeared on the 2nd to 9th day after *Porphyra* was exposed to the atmosphere for more than 4 hours and, at the same time, the difference in temperature exceeded 8°C. Then the number of white and dead cells increased markedly and plants died 4 days after the appearance of *Beneckea*. However, there were a few cases in the past three fiscal years where *Beneckea* populations were not found on the thalli even under the appropriate environmental conditions. It is not certain that *Beneckea* requires the repetition of the above mentioned conditions or only that the environmental factors must include these.

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ノリの微生物学的研究—V

ノリの病害と細菌との関連について

月　舘　潤　一

前報でノリに白色死細胞が増加すると，それと並行して特定のバクテリヤが出現すると報告したが，この関連を更に確かめるため，養殖ノリ及び岩に着生して生長するノリについて，昭和48年度，49年度及び50年度の3ケ年間にわたって，調査，観察を行った。

いずれの年度もノリに白色死細胞が増加すると，*Beneckea* が出現した。昭和48年度は *Beneckea* が出現した日に白色死細胞が増加し，49年度は *Beneckea* が出現した3日後に，50年度は2日後及び4日後に白色死細胞が増加した。しかも，50年度はかなりの葉体が消失した。このときの経過は須藤らの記載による凝似しろくされ症に類似していた。従って，凝似しろぐされ症の原因の一つはバクテリの *Beneckea* によって生ずると考えられた。

Beneckea が出現した原因を検討するため，漁業者の間に明け方に干出があって，そのときの温度差が大きいと病害が出るとの観察があるので，干出時間と干出前後の温度差を調べた。昭和48年度は *Beneckea* が出現した5日前に干出時間が4時間以上あり，その前後の温度差が9.8℃で，49年度は *Beneckea* が出現した7日前に干出が4時間以上あって，温度差が9.5℃で，50年度は *Beneckea* が出現した2日，及び9日前に干出が4時間以上あり，その温度差が8.4℃であった。

つまり，干出時間が4時間以上あり，このときの温度差が8.0℃以上になると，その2～9日後に *Beneckea* が出現し，そして更にその4日後までに白色死細胞が増加して，50年度ではかなりの葉体が消失した。

しかし，干出時間，温度差の条件がみたされても，*Beneckea* は出現しないときが各年度でみられ，この原因は明らかにし得なかった。