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Isolation of Algophorous *Labyrinthula* and Amoebae from Marine Habitats

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Keywords : *Labyrinthula*, Amoeba, Plaque, Macroalgae, Eelgrass.

Abstract

Labyrinthula and amoeba spp. were isolated from marine habitats, especially the surface of the thallus of marine macroalgae and eelgrass by using double layer agar plates containing *Chaetoceros* cells. They formed the characteristic plaques on double layer agar plates by digesting the diatom cells. *Labyrinthula* isolates formed plaques with concentric circle lines and showed a variety of lag time of plaque growth on the same agar plate. On the other hand, plaques produced by an amoeba strain were relatively uniform in diameter but growth rates of plaques varied among amoeba strains. Both organisms were found to grow as colonies on marine agar plates supplemented with either cell debris or cell extract from some bacteria or yeasts, or animal sera.

A marine slime mold, *Labyrinthula* and a diverse group of protozoa, amoebae are known to digest a variety of seaweeds and seagrasses. Especially, marine *Labyrinthula* spp. were identified as the primary microorganisms causing the wasting disease of eelgrass, *Zostera marina*.[1-4] *Labyrinthula* cells have been thought to make small wound openings on the surface of the leaves by enzymatic degradation and then to enter the leaves through the wound openings. On the other hand, most of the marine amoebae are believed to feed on bacteria, microalgae, decaying plants, and animal matter.[5-7] We have also isolated marine *Labyrinthula* and amoebae spp. from sea water samples by use of double layer agar plates containing diatom cells.[8]

In this paper, we isolated marine algophorous *Labyrinthula* and amoebae from sea water samples including marine microalgae, macroalgae, and seagrasses, and studied on growth response of these microorganisms on agar plates.

Materials and Methods

Isolation of algophorous microorganisms

Sea water, macroalgae and seagrass samples were collected from coastal area of Kagoshima Bay and transported to the laboratory in an ice box. Sea water and microalgal samples were mixed with soft agar containing ESS medium and *Chaetoceros ceratosporum* cells, and layered on basal agar plates. Macroalgae and seagrass samples were cut into 2×4㎜ sized pieces and then 5 pieces were put on a double layer agar plate containing diatom cells.

Culture of diatom cells

Chaetoceros ceratosporum, which was provided by Dr. Fukami of Kochi University, was usually cultivated in 300 ml of Provasoli's enrichment sea water medium (ESS) with aeration under illumination of 5,000 lx (12L: 12D light cycle). Diatom cells were harvested with centrifugation after 7-10 days of incubation and then resuspended in fresh ESS medium at a density 10 times higher than the original. Double layer agar plates were prepared by mixing

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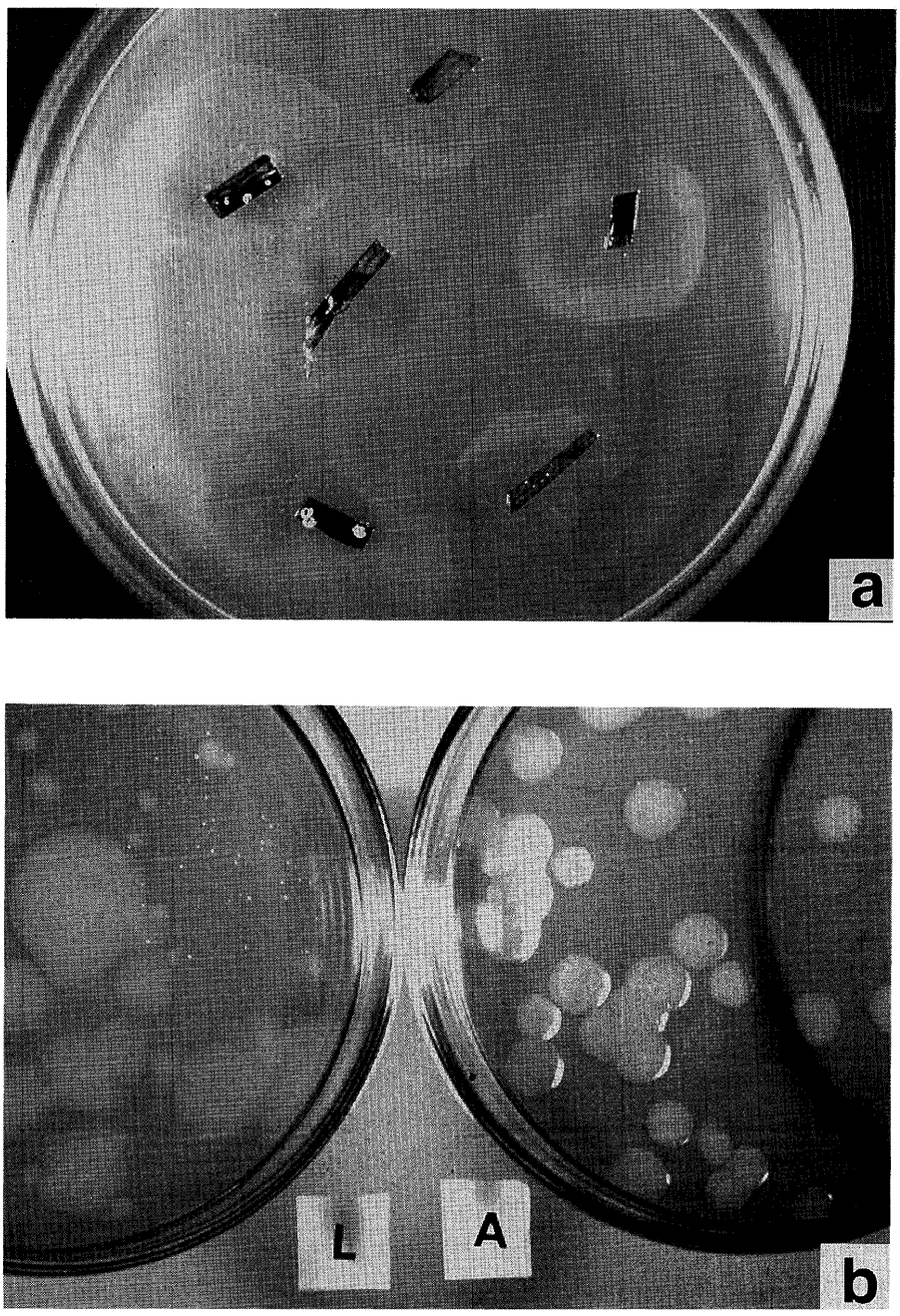

Fig. 1 Plaque and cell morphology of *Labyrinthula* and amoeba isolates.
a, plaque formation around eelgrass pieces on a double layer agar plate;
b, plaque morphology of *Labyrinthula* (L) and amoeba (A) isolates;

one ml of the diatom cell suspension with 2 ml of ESS soft agar (0.8% of agar concentration) maintained at 50℃ to be poured on an ESS basal agar plate as described in previous papers.[9-11]

Culture of bacteria and yeast strains

Bacteria and yeast strains examined were grown at 30℃ on a ZoBell 2216E modified medium (Z-Cll medium) and ZAG agar plates, respectively. Baceria or yeast cells obtained were collected from 10 agar plates, washed, and resuspended in 20 ml of ESS liquid medium.

Preparation of bacteria and yeast cell extracts

Bacteria and yeast cell suspensions (10 ml) were autoclaved and then filtrated through a membrane filter (Millipore filter, pore size 0.22 μ m). Cell extract or cell debris suspension autoclaved (10 ml) were

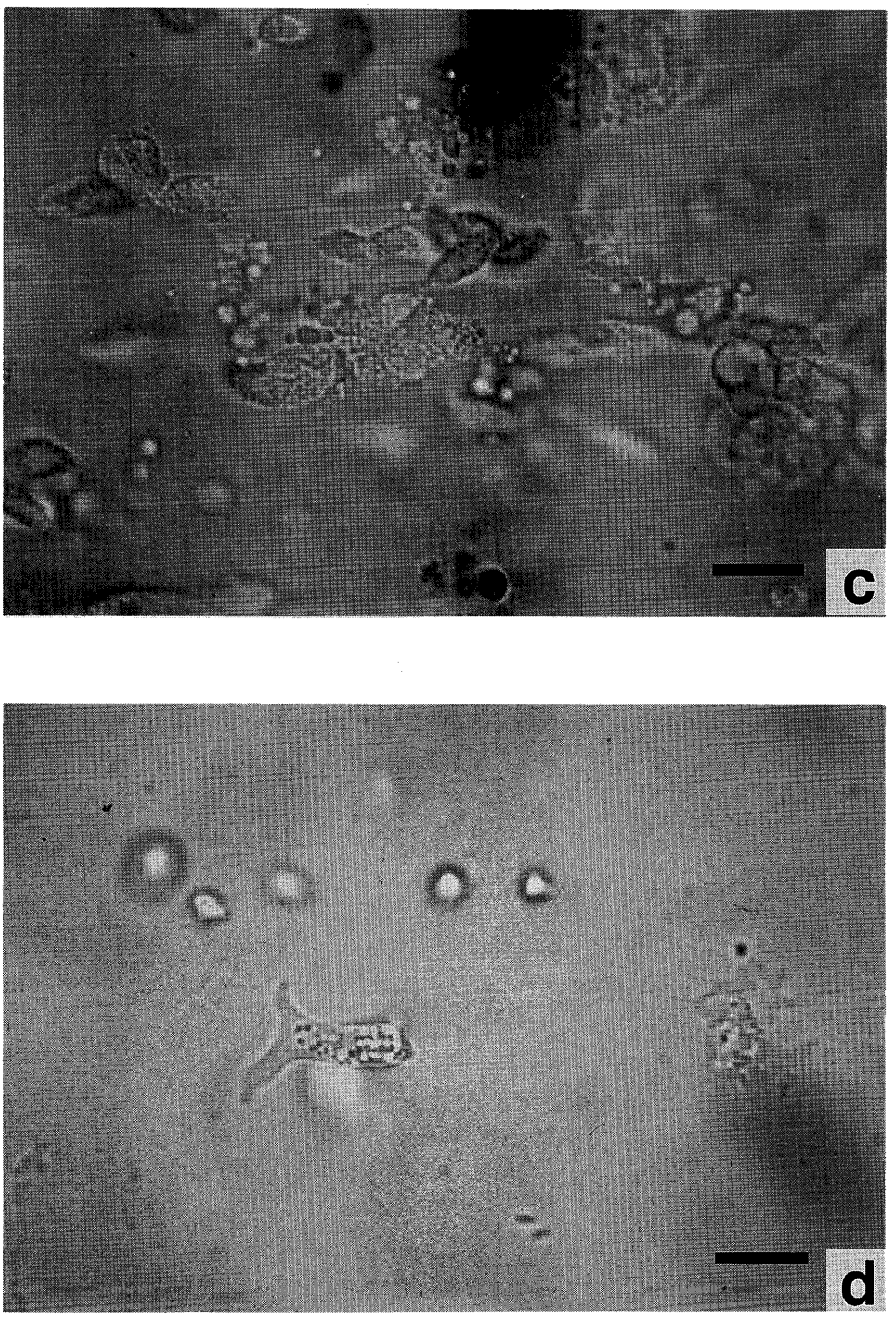

Fig. 1 Plaque and cell morphology of *Labyrinthula* and amoeba isolates.
c, light micrograph of *Labyrinthula* cells;
d, light micrograph of amoeba cells, bar indicates 10 μ m.

added to 90 ml of ESS agar medium to be poured into petri dishes. Bovine or horse sera were also mixed with ESS agar medium at 1% concentration to make agar plates in the same process.

Algicidal activity

Digestive zone around colonies of test organisms or plaques appearing on double layer agar plates with diatom cells or test organisms were measured in diameter every day during incubation of the agar plates at 23℃ under illumination.

Results

Isolation of Labyrinthula and amoebae from sea water, macroalgae and seagrass

Plaque-forming microorganisms were isolated from sea water samples by the double layer agar

Table 1 Isolation of algicidal microorganisms from sea water samples

Number of sampling site	Number of plaque per plate					T.C.C*1
	Large	Medium	Small	Tiny	Log No./ml	Log No./ml
1 Shrimp pond (zoea)	2+*2	6+	26	516	4.7	4.9
2 Shrimp pond (zoea)	1@*3	13+	122	17	4.2	4.7
3 Shrimp pond (adult)	0	1+	2	2	2.7	4.7
4 Shrimp pond (adult)	0	1+	6	4	3.0	4.6
5 Shrimp pond (adult)	3+	0	1	2	2.8	4.9
6 Flatfish pond (adult)	0	0	5	1	2.8	5.0
7 coastal water (1)	0	1+	1	0	2.3	3.7
8 coastal water (2)	0	1+	1	0	2.3	2.6
9 coastal water (3)	0	1+	1	0	2.3	4.7
10 coastal water (4)	1+	0	0	0	2.0	4.9
11 coastal water (5)	0	0	0	0	0.0	4.9

*1, total colony count.
*2, + indicates isolation of algicidal filamentous bacteria.
*3, @ indicates isolation of amoeba.

Table 2 Isolation of algicidal microorganisms from eelgrass

Microorganism	Number of isolate/sample	
	7/13/'95	7/5/'96
Labyrinthula	18/45	10/10
Amoeba	1/45	0/10
Filamentous bacteria	39/45	0/10

Table 3 Isolation of algicidal microorganisms from macroalgae

Macroalga	*Labyrinthula*	Amoeba	Fil. bacteria*1
11/2/'95			
"Shiogusa"*2	4/4	0/4	1/4
"Hondawara"	0/6	2/6	2/6
"Jyuzumo"	4/4	0/4	0/4
"Aosa"	0/4	0/4	1/4
"Tengusa"	1/2	0/2	1/2
"Umizomen"	1/4	0/4	2/4
7/5/'96			
"Endoumoku"	2/10	2/10	3/10
"Futaemoku"	0/10	1/10	0/10
"Sujiaonori"	8/20	4/20	11/20
"Aosa"	0/20	2/20	15/20
"Tengusa"	2/10	7/10	6/10

*1, filamentous bacteria.
*2, "Shiogusa", *Cladophora* sp.; "Hondawara", *Sargassum fulvellum* ; "Jyuzumo", *Chaetomorph* sp.; "Aosa", *Ulva pertusa* ; "Tengusa", *Gelidius amansii* ; "Umizomen:, *Nemalion vermiculare* ; "Endoumoku", *Sargassum yendoi*; "Futaemoku:, *Sargassum duplicatum* "Sujiaonori:, *Enteromorpha prolifera*.

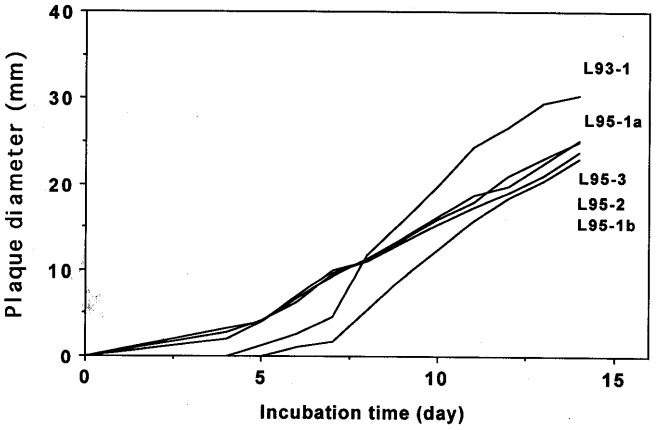

Fig. 2 Plaque growth of *Labyrinthula* strains on double layer agar plates. Data present average of plaque diameter for each strain.

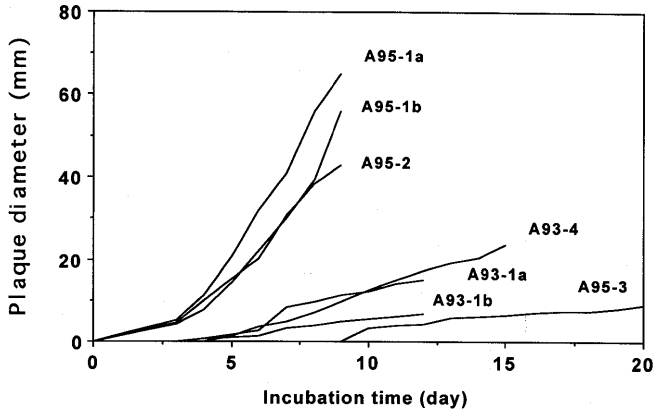

Fig. 3 Plaque growth of amoeba strains on double layer agar plates.
Data present average of plaque diameter for each strain.

method as shown in Tables 1, 2, and 3. When plaque zones appearing on the agar plates were picked up into a glass slide and observed under a microscope, it was found that the large- and medium- sized plaques were produced exclusively by *Labyrinthula*, amoebae or filamentous bacteria. On the other hand, the small- and tiny-sized plaques were caused mostly by rod-shaped bacteria. Therefore, we tried to isolate *Labyrinthula* and amoeba strains from the large- and

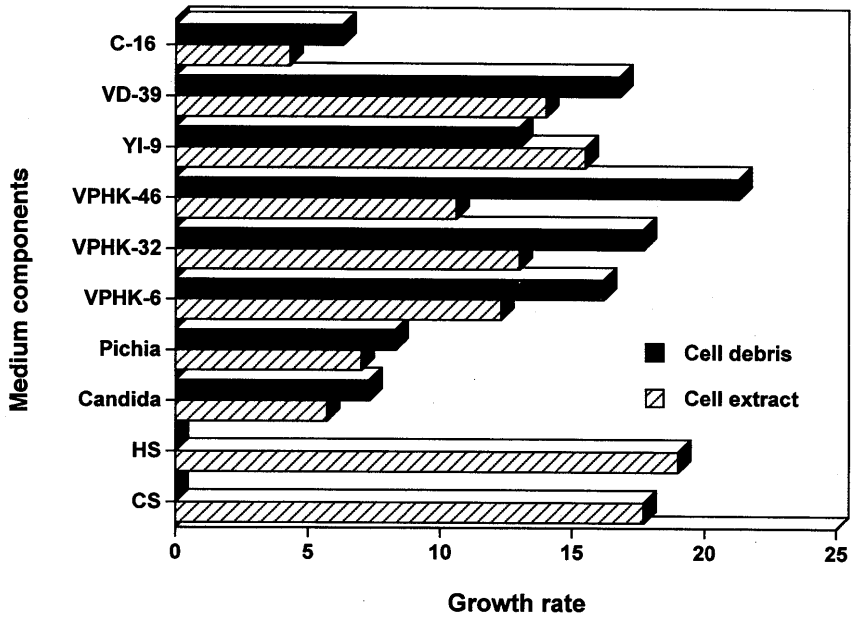

Fig. 4 Colony growth of *Labyrinthula* L95-1 on agar plates supplemented with cell debris, cell extract, or serum.
C-16, marine *Pseudomonas* sp.; VD-39, YI-9, VPHK-46, VPHK-32, VPHK-6, marine *Vibrio* spp.; HS, horse serum; CS, bovine serum.
Growth rate indicates the ratio of colony diameter to inoculated sample diameter.

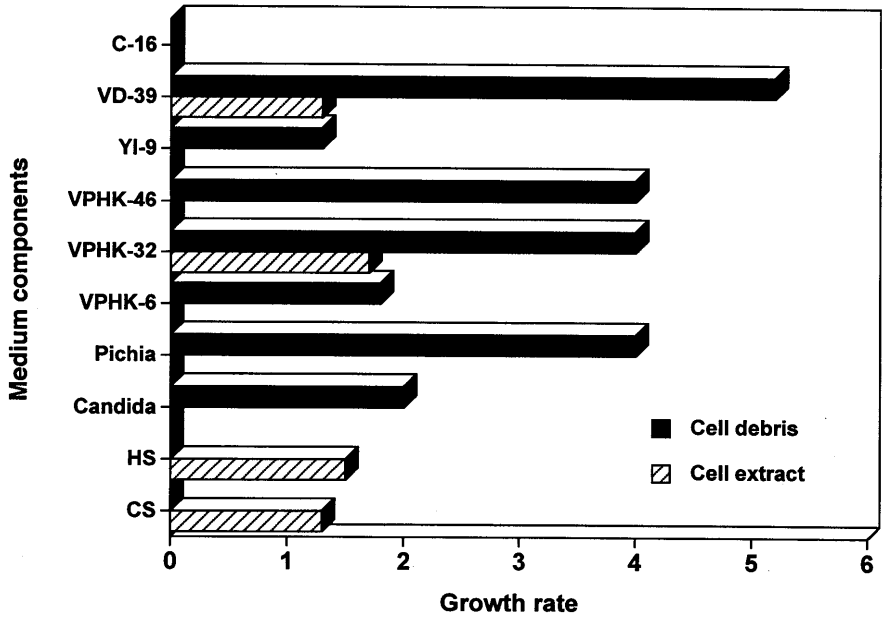

Fig. 5 Colony growth of amoeba A95-3 on agar plates supplemented with cell debris, cell extract, or serum. Abbreviations are the same as in Fig. 4.

medium-sized plaques. In fact., we succeeded in isolating algicidal *Labyrinthula*, amoeba and *Saprospira* strains from the sea water samples of juvenile shrimp culture ponds, in which various kinds of diatoms were cultured artificially in high density in order to stabilize and maintain water quality of the shrimp culture ponds.

As shown in Fig. 1, and Tables 2 and 3, various kinds of plaques occurred around most of the sample pieces of marine macroalgae or seagrass put on the double layer agars. We could isolate many *Labyrinthula*, amoeba, and *Saprospira* strains from

the plaques appeared around marine algal or seagrass samples.

Plaque growth of Labyrinthula and amoebae

Fig.1b illustrates plaque morphology of *Labyrinthula* and amoeba strains on the double layer agar plates. *Labyrinthula* and amoeba strains formed plaques with a characteristic morphology. For example, plaques formed by amoeba strains possessed the translucent inner area and relatively distinct boundary line, while *Labyrinthula* strains produced plaques with concentric circle lines. Some plaques appeared at early incubation time (4 days) and some plaques were visible at late incubation time (6-7 days) on one agar plate inoculated by a *Labyrinthula* strain as shown Figs. 1b and 2. Plaques produced by an amoeba strain were relatively uniform in diameter on one agar plate but growth rates of plaques varied among amoeba strains as shown in Figs. 1b and 3.

In Figs. 1c and 1d, light micrographs of cells of *Labyrinthula* and amoeba are presented. Spindle-shaped cells of *Labyrinthula* gathered to form cell aggregates with membrane-like structure, ectoplasmic network. On the other hands, single cells of amoeba. moved individually with pseudopod protrusions in the direction of movement.

Growth of Labyrinthula and amoebae on agar plates containing bacteria or yeast extracts

Colony growth of *Labyrinthula* and amoeba strains on agar plates containing either cell debris or cell extracts from bacteria or yeast strains are shown in Figs. 4 and 5. *Labyrinthula* strain L95-1 grew very well as colony on agar plates including bovine serum, horse serum, cell debris, or cell extracts of marine *Vibrio* strains, while it grew slowly on agar plates containing yeast cell extracts. On the other hand, amoeba strain A95-3 grew better as colony on agar plates containing cell debris from *Vibrio* strains, but showed a little growth on agar plates containing cell extracts or serum.

Discussion

Almost all aquatic plants including macroalgae, seagrass have epiphytic microorganisms which are usually very dense. The dominant organisms of epiphytes are known to be various kinds of bacteria, fungi and diatoms. These microorganisms are thought to affect the host plants by exchange of their photosynthetic products, antibiotics or growth stimulating substances.[12-14]

Labyrinthula spp. are a kind of slime mold, which was reported to be the primary microorganisms causing the wasting disease of eelgrass (*Zostera marina*). They may also be pathogenic on some marine macroalgae. They were suggested to invade eelgrass leaves through small wound openings on the surface of the tissue by enzymatic degradation. The plant cell walls were observed to be degraded by the association of ectoplasmic network with degraded plant cell walls. On the other hand, amoebae are a diverse group of protozoa, which are believed to feed on detritus, bacteria, decaying plant, or microalgae.

We isolated many strains of *Labyrinthula* and amoebae from marine environments, especially the thallus of marine macroalgae and eelgrass by using double layer agar plates containing *Chaetoceros* cells. They formed the characteristics plaques or plaque-like clear zones on the double layer agar containing diatom cells. Furthermore, they could grow as colonies on agar plates supplemented with either cell debris or cell extract of some bacteria or yeasts, or animal sera. These facts indicate that *Labyrinthula* and amoebae can utilize whole cells or cell components from diatoms, yeasts, or bacteria for their growth.

In marine environment, *Labyrinthula* and amoeba are considered to live on the surface area of benthic sea plants or macroalgae to obtain nutrients by predating or lysing epiphytic microorganisms including diatoms, yeasts and bacteria. The degradation mechanism of prey organisms and essential nutrients for predator organisms remain to be studied.

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