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Studies on the marine yeasts. V
Yeasts isolated from seaweeds

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A considerable development of yeasts was observed in some seaweeds rotted in a flask. But the growth rate of the yeasts was low, and the number of the yeasts became maximum after two to four weeks storage at 20°C. There were many protozoa in the flask containing the rotted seaweeds, and the yeasts seemed to be the food of the protozoa. Without the protozoa, far more yeasts could have been observed and the growth rate of yeasts could have been higher than in the presence of the protozoa. When chlortetracycline and citric acid were added to the seaweed stored in a flask, no protozoa was observed and far more yeasts were detected in the storage than in the seaweeds not treated, and the number of the yeasts became maximum after seven days storage. As is shown in the previous report,[1] no yeasts were detected in seaweeds belonging to *Rhodophyta* rotted in a flask, and there were many seaweeds belonging to *Chlorophyta* and *Phaeophyta* in which no yeasts were detected when the seaweeds were rotted in a flask. But by the treatment with the two compounds mentioned above, many yeasts were always observed in rotted seaweeds irrespective of the species of the seaweeds tested and date of collection.

EXPERIMENTAL

About 15–20 g of seaweeds was picked at several places on the beach of Tsuyazaki and put into a sterilized cotton-plugged flask and transferred to the laboratory. Then 200 ml of sea water obtained at the same place was poured to the flask, and the flask was stored at 20°C after the addition of chlortetracycline (50 p.p.m.) and citric acid (200 p.p.m.).

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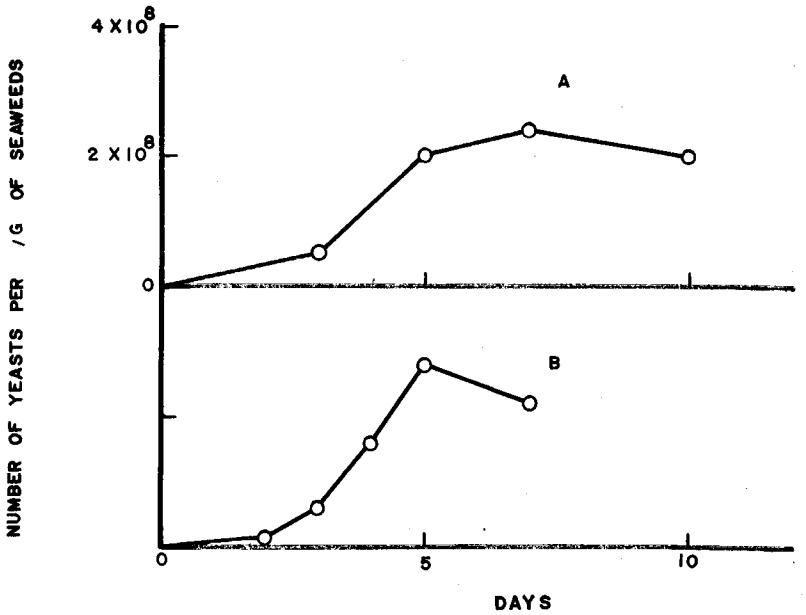

Fig. 1. Number of yeasts developing in 1g of *Chlorophyta* stored at 20°C after addition of chlortetracycline and citric acid.
A: *Ulva pertusa*, B: *Enteromorpha* sp.

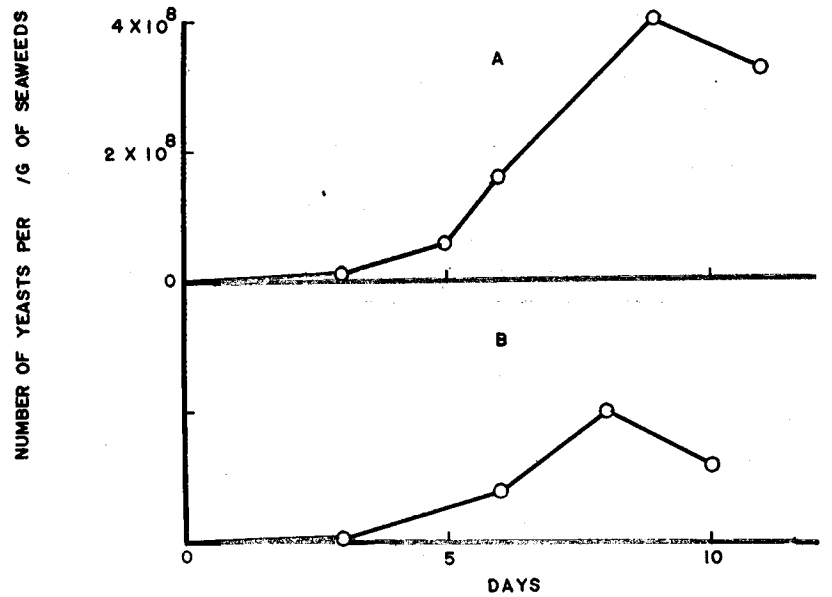

Fig. 2. Number of yeasts developing in 1g of *Rhodophyta* stored at 20°C after addition of chlortetracycline and citric acid.
A: *Hyalosiphonia caespitosa*, B: *Polysiphonia urceolata*

The flask was sampled at 24 hr. intervals during 240 hr. storage period. The method used for the isolation of yeasts and taxonomic procedures used were similar to those in the previous reports.[2,3]

The number of the yeasts developing in the stored seaweeds is given in Figs. 1, 2 and 3. The number varied according to the species of seaweeds and appeared to vary with the date of collection.

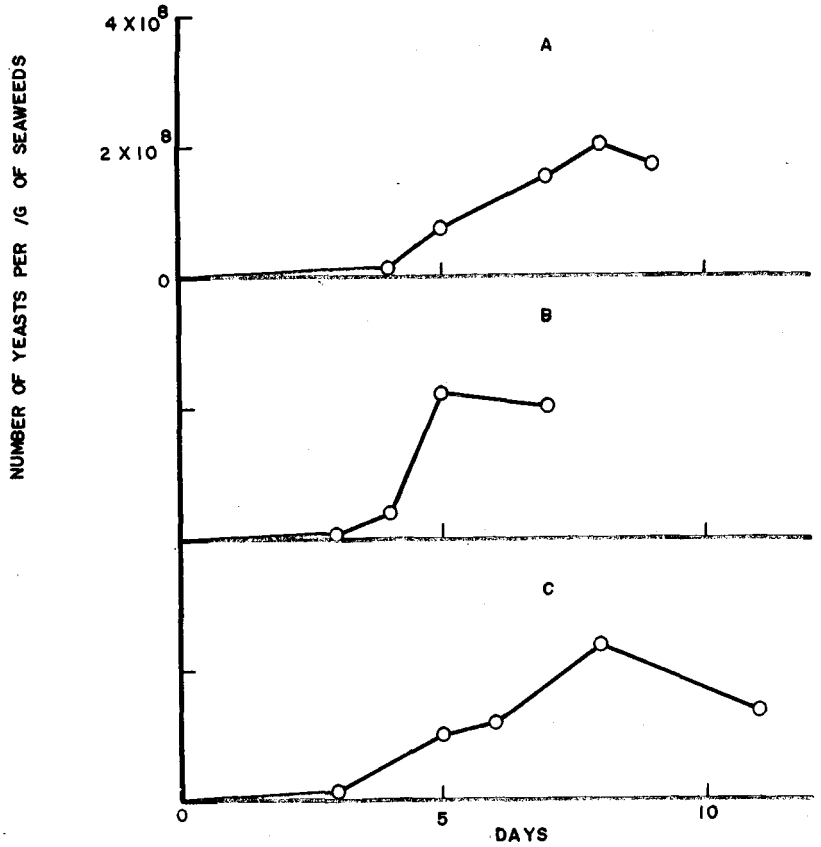

Fig. 3. Number of yeasts developing in 1g of *Phaeophyta* stored at 20°C after addition of chlortetracycline and citric acid.
A: *Sargassum* sp., B: *Sargassum thunbergii*, C: *Scytosiphon lomentaria*

Since the protozoa attaching themselves to seaweeds may give the yeasts their nutrients, a seaweed was washed with detergent to eliminate the protozoa. Then chlortetracycline and citric acid were added both to the washed and unwashed seaweed, and they were stored at 20°C. The results are given in Fig. 4. The washing did not affect the number of the developing yeasts, but the growth rate of the yeasts was affected

by it. It seems that as most of the yeasts attaching themselves to the seaweed were washed away, the growth rate became low.

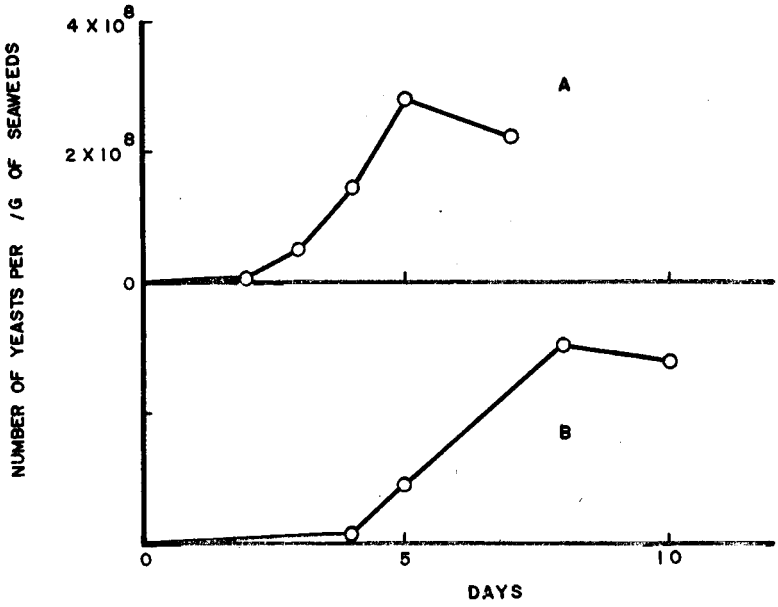

Fig. 4. Effects of washing seaweed on the number and the growth rate of developing yeasts. Seaweed tested was *Enteromorpha* sp.
A: Unwashed, B: Washed

Thirty-nine cultures of yeasts were isolated from the stored seaweeds, and their taxonomic positions are given in Table 1.

Table 1. Yeasts collected from rotted seaweeds.

Taxon	Number of isolates
Rhodotorula mucilaginosa	8
Cryptococcus albidus	1
Cr. diffluens	1
Cr. laurentii	2
Cr. neoformans	1
Candida tropicalis	2
C. parapsilosis var. *intermedia*	9
C. brumptii	1
C. tenuis	2
C. sp.	3
Trichosporon cutaneum	1
Tr. infestans	1
Torulopsis famata	7

In *Chlorophyta*, developing yeasts were similar to those in the case of plankton. Namely, during the first two days storage, the

developing yeasts were *Rhodotorula* (90 %) and *Cryptococcus* (10 %). After seven days, *Candida* and *Torulopsis famata* predominated and the rest was *Rhodotorula*. The species of *Candida* were *C. tropicalis* and *C. parapsilosis* var. *intermedia*.

In *Rhodophyta*, during the first two days storage *Rhodotorula* was only 5 %, and after seven days almost all of the developing yeasts were *C. parapsilosis* var. *intermedia*.

Excepting in *Colopomenia bullosa*, no *Rhodotorula* was detected in *Phaeophyta* in all stages of the storage. In *Col. bullosa*, during the first two days, the yeasts were *Cryptococcus* (50 %), *Rhodotorula* (20 %) and *Candida* (30 %), and after four days almost all of the yeasts were *C. parapsilosis* var. *intermedia*. In other *Phaeophyta* almost all of the yeasts were invariably *C. parapsilosis* var. *intermedia*.

Discussion

In general, only bacteria are observed in plankton and seaweeds, rotted in a flask, and putrefactive odour is remarkable. But, as previously mentioned, a remarkable development of yeasts was observed in some seaweeds and *Thalassiosira subtilis* (marine diatom),[2] rotted in a flask, and no putrefactive odour was observed. When plankton or seaweeds were emitting putrefactive odour, no yeasts could be detected. So it appears that the growth of yeasts are checked by certain metabolic products of bacteria. When chlortetracycline and citric acid were added to inhibit bacterial growth, yeasts were always remarkably detected in stored plankton or seaweeds. Since in the sea there is not sufficient concentration of the metabolic products to check the growth of yeasts, yeasts may grow in decaying plankton and seaweeds there. This idea is supported by the fact that, as is given in the previous report,[2] the number of yeasts, detected in *Thalassiosira subtilis* on the day of collecting, was 0–10 per ml in February and March when the organism is vigorous in the sea, but in April, when the organism begins to decay, the number was 100–200 per ml.

As previously mentioned, members of *Rhodotorula* and *Cryptococcus* were dominantly detected in the treated seaweeds and plankton, stored in a flask, in the early stage of storage, and in the last stage the developing yeasts were *Candida*, especially *C. tropicalis*, *C. parapsilosis* var. *intermedia* and *C.* sp. This fact substantiates the observations of Fell *et al*[4] who isolated these species of yeasts except *C.* sp. from the ocean. And ZoBell[5] reported that yeasts seemed to be generally present regardless of the distance from land, and some samples of the ocean contained more yeasts than bacteria. Those reports seem to support the above mentioned idea that yeasts in the sea may grow

in decaying seaweeds and plankton. But the rôle of yeasts in the cycle of life in the sea may differ considerably from that of bacteria. Namely, when the same sample of plankton was decomposed by bacteria and yeasts respectively, the volume of all the developing yeasts was more than fifteen times larger than that of the bacteria.[5] Further work in progress ivolves the investigation to reveal what part of the constituents of plankton and seaweeds the yeasts assimilate.

In general, yeasts are not parasitic but saprophytic. Thus in the sea, living plankton or seaweeds may not be decomposed directly by the yeasts. When plankton or seaweeds fade and begin to decay, the yeasts may grow in them. It appears that even at that time, they are decomposed by the yeasts not directly but after their autolysis.[6] Of course, some parts of cell constituents of them may be decomposed directly by the yeasts.

Bacteria themselves have been shown as sources of food for animals. One of the authors has shown that yeasts were also the sources of food for animals (protozoa).[7] And as in the case of bacteria, yeasts may give themselves to other animals as sources of food.

The abundance of *Candida tropicalis* (in summer collection) agrees with the observations of Fell *et al*[4] and Bhat *et al*,[8] but the development of this species was rare in winter collection. It may be due to the difference of temperature of sea water. Three cultures of the species isolated in summer were able to grow at 41°C, but all culture of it isolated in winter were unable to grow even at 37°C. And the species of *Candida*, developing in rotted plankton in the last stage of storage, varied according to the seasons. Therefore it seems that the yeast flora in the sea vary seasonally.

SUMMARY

1) A remarkable development of yeasts was always observed in the storage of seaweeds that were stored for several days at 20°C after the addition of chlortetracycline and citric acid.

2) The number of the developing yeasts varied according to the species of the seaweeds tested and it was from 4×10^7 to 4×10^8 per 1g of wet seaweeds.

3) Thirty-nine cultures of yeasts were isolated from the stored seaweeds (15 species) : 8 of the cultures were members of the genus *Rhodotorula*, 5 of *Cryptococcus*, 17 of *Candida*, 2 of *Trichosporon*, and 7 of *Torulopsis*.

4) In *Chlorophyta*, during the first two days storage, *Rhodotorula* (90 %) and *Cryptococcus* (10 %) predominated, and after seven days *Candida parapsilosis* var. *intermedia* and *Torulopsis famata* were dominantly

detected.

In *Rhodophyta, Rhodotorula* were only 5 % during the first two days, and after seven days almost all of the yeasts were *C. parapsilosis* var. *intermedia.*

Excepting in *Colopomenia bullosa,* no *Rhodotorula* was detected in *Phaeophyta* in all stages of the storage. In *Col. bullosa,* during the first two days, the yeasts were *Cryptococcus* (50 %), *Rhodotorula* (20 %) and *Candida* (30 %), and after four days almost all of the yeasts were *C. parapsilosis* var. *intermedia.* In other *Phaeophyta* almost all of the yeasts were invariably *C. parapsilosis* var. *intermedia.*

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