Studies on the marine yeasts. IV : Yeasts isolated from marine plankton

Suehiro, Sumio
Laboratory of Chemistry of Fishery Product, Faculty of Agriculture, Kyusyu University

Tomiyasu, Yukio
Laboratory of Chemistry of Fishery Product, Faculty of Agriculture, Kyusyu University

Tanaka, Otohiko
Fisheries Laboratry, Department of Agriculture, Kyusyu University

https://doi.org/10.5109/22698

出版情報：九州大学大学院農学研究院紀要. 12 (3), pp.155-161, 1962-11. Kyushu University
バージョン：
権利関係：

Journal of the Faculty of Agriculture, Kyushu University, Vol. 12, No. 3
November 30, 1962

Studies on the marine yeasts. IV
Yeasts isolated from marine plankton

SUMIO SUEHIRO,* YUKIO TOMIYASU† and OTOHIKO TANAKA†

There are very few reports referring to the biology of the yeast population in the ocean,[1,2,3] and nothing has ever been revealed about the rôle of the yeasts in the cycle of life in the sea.

In one of the authors' experiment,[4] the yeasts developed in some seaweeds (belonging to *Chlorophyta* and *Phaeophyta*) stored in a flask in their fading period. The number of the developing yeasts became maximum after two to four weeks storage. It varied according to the species and date of collection, and it was 2,000–160,000 per 1 g of wet seaweeds. Six species of yeasts were isolated from the stored seaweeds, and their taxonomic positions were determined as follows: *Candida albicans*, *C. parapsilosis* var. *intermedia*, *Trichosporon behrendii*, *Tr. cutaneum*, *Tr. infestans* and *Torulopsis famata*. The cultures of *Tr. behrendii* and *T. famata* were able to grow on wort agar (10° Balling.) containing 20 % NaCl.

One of the authors reported[5] that many yeasts had been observed in *Thalassiosira subtilis* (marine diatom) rotted in a flask and the number of the developing yeasts had been about 2×10^7 per 1 g of the organism. Sixteen cultures of yeasts were isolated from the rotted organism and six of them were found to belong to *Candida parapsilosis* var. *intermedia*, three to *C. lipolitica*, three to *Cryptococcus laurentii*, three to *Torulopsis inconspicua* and one to *Rhodotorula mucilaginosa*. Putrefactive odour was observed in neither case. But when seaweeds decayed with a bad smell, no yeasts were detected in them.

There were some cases in which many yeasts developed in the plankton decayed in a flask and no putrefactive odour was detected. But in general, when plankton was decayed in a flask, only bacteria were observed to develop and putrefactive odour was remarkable.

* Fishery Research Laboratory, Kyushu University, Tsuyazaki, near Fukuoka.
† Department of Fisheries, Kyushu University, Fukuoka.

The growth of the yeasts seems to be prevented by certain metabolic products of bacteria. To check the bacterial growth, therefore, chlortetracycline and citric acid were added[6] to the plankton collected. It was found that many yeasts were always observed in the treated plankton after several days storage.

MATERIALS AND METHODS

Collections of marine plankton were made at two stations (Fig. 1). Zooplankton was collected with a zooplankton-net the entrance of which was covered with a net (1 mm meshes) to prevent medusa from entering. Phytoplankton was collected with a phytoplankton-net the entrance of which was covered with a bolting silk (G.G. 54) to prevent zooplakton from entering. But it is impossible to prevent a small amount of zooplankton larvae with the same size as phytoplankton from entering. At the collection station, a bucket of the plankton-net was opened and the net was thoroughly washed with sea water to check the contamination by terrestrial yeasts and then the bucket was shut and the net was towed at 10 m depth at one knot for five minutes. As soon as the net was brought to the surface, the collected plankton was transferred directly to a sterilized cotton-plugged flask from the net. The

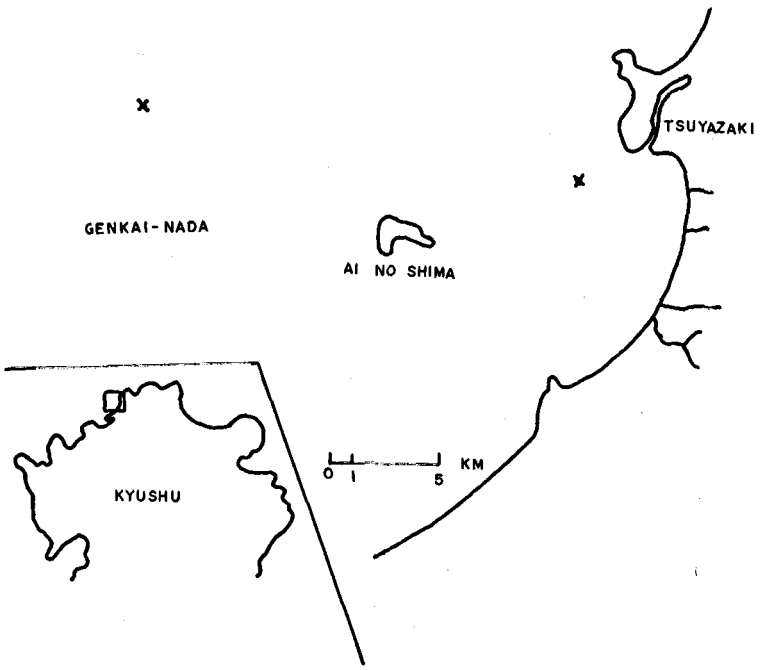


Fig. 1. Map of the part of Genkai-nada, Fukuoka, with collection stations.

flask was transported to the laboratory, and it was stored at 20°C after the addition of chlortetracycline (50 p.p.m.) and citric acid (200 p.p.m.).[6]

The flask was sampled at 24 hr. intervals during 240 hr. storage period. The isolation medium for the yeasts consisted of 3% Malt Extract (Difco) and 1.5% agar, and was prepared with sea water. Suitable dilutions were inoculated to the medium to which chlortetracycline was added just before inoculation. Replicate platings were made at each sampling, and the plates were incubated at 20°C for seven days, and the colonies were picked to agar slants. Taxonomic procedures used were similar to those of Lodder and Kreger van-Rij.[7]

Experimental Results

Many yeasts were always observed in the stored plankton (Figs. 2, 3).

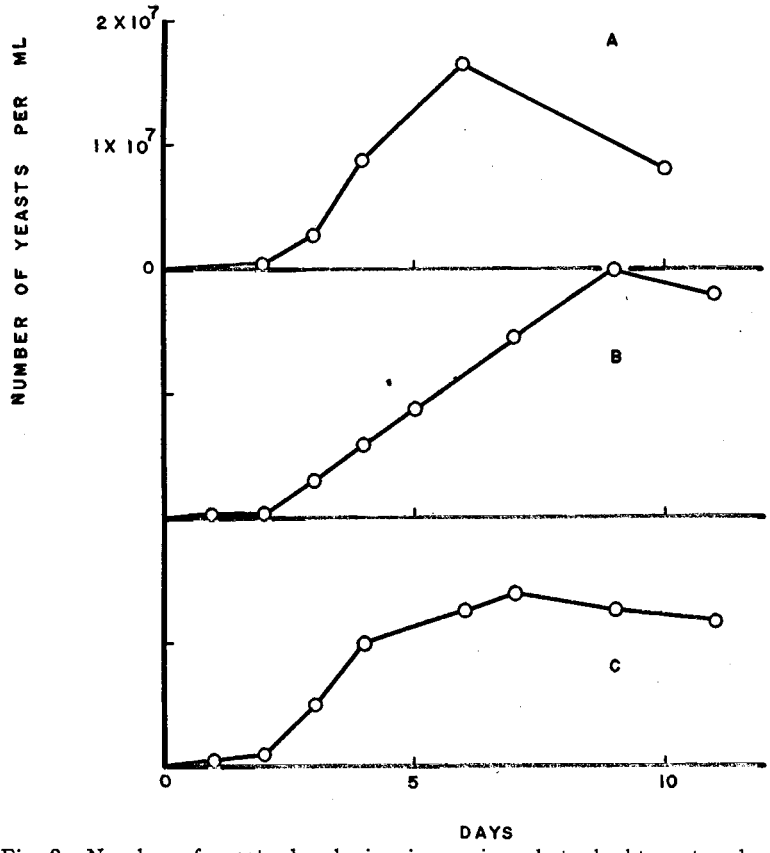


Fig. 2. Number of yeasts developing in marine phytoplankton stored at 20°C after addition of chlortetracycline and citric acid.
A: Collected on 28th, June, B: 28th, August, C: 17th, October.

The maximal number of the yeasts were obtained after about seven days storage. The number of them was affected by the volume and the species of plankton and it was proportional to the volume of plankton in the same collection. Because the volume of the tested plankton was too small to measure their weight exactly, it was impossible to estimate how many yeasts developed from 1 g of the plankton. Then, for this estimation, it was attempted to collect sufficient weight of phytoplankton or zooplankton. Since the size of zooplankton cells is generally larger than that of phytoplankton, it is possible to collect only zooplankton from marine population by filtration with net. But it is impossible to collect phytoplankton only, because as previously mentioned, even if the entrance of net is covered with a bolting silk to prevent zooplankton from entering, zooplankton larvae with small size (the same size as phytoplankton) are able to

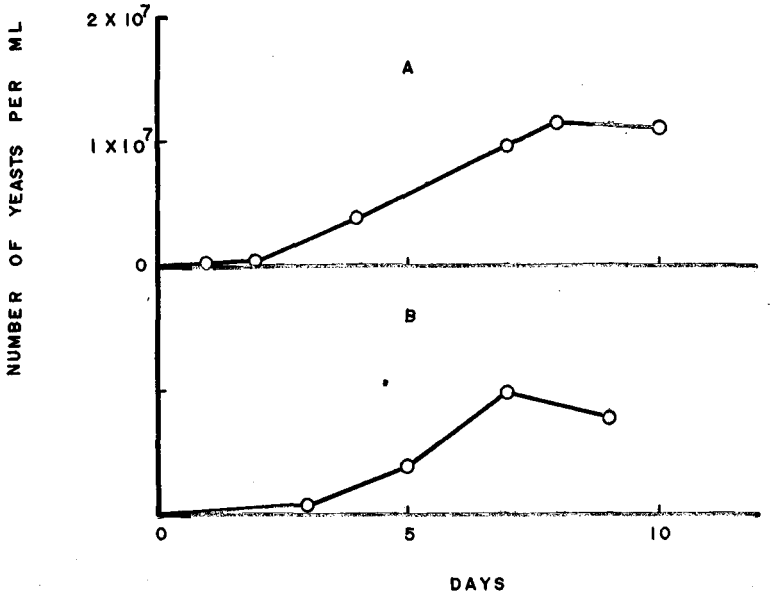


Fig. 3. Number of yeasts developing in marine zooplankton stored at 20°C after addition of chlortetracycline and citric acid.
A: Collected on 17th, November, B: 14th, December.

pass through the covering. Therefore, in order to measure how many yeasts develop from 1 g of phytoplankton, it is indispensable to culture it. Accordingly one cell of a certain sort of phytoplankton was isolated and cultured purely. The method used was as follows: the mixture of inorganic salts*[8] was added to the flask containing sea water

* KH_2PO_4 . . 4 mg (as P_2O_5) KNO_3 . . 28 mg (as N) K_2SiO_3 . . 5 mg (as SiO_2)
$FeCl_3$ . . . 1 mg (as Fe) sea water 1,000 ml.

collected newly. The flask was placed near a window on the north side. After two weeks, a considerable amount of phytoplankton was observed in the enriched sea water. Then a few drops of it were put on a hole object glass and the glass was placed on the stage of a stereoscopic microscope. The one cell of the developing phytoplankton was picked with a sterilized capillary tube (dia. 120–150μ) to a drop of sterilized sea water on another sterilized hole object glass. Then after one minute the latter was placed on the stage of the stereoscopic microscope, and the isolated cell was picked again to a drop of sterilized sea water on another hole object glass. After the manipulation was made six times, the washed cell of phytoplankton was inoculated to the flask containing enriched sea water, and it was incubated at room temperature near a window on the north side. After two weeks incubation, remarkable development of the phytoplankton was observed in the flask.

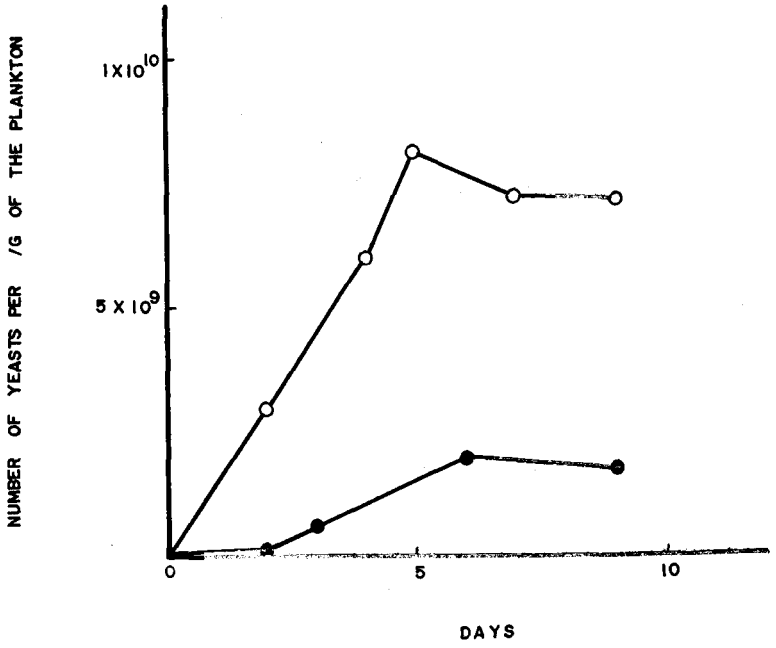


Fig. 4. Number of yeasts developing in 1 g of pure cultured phytoplankton and of *Doliolum* sp. both stored at 20°C after addition of chlortetracycline and citric acid. —○—: phytoplankton, —●—: *Doliolum* sp.

Then chlortetracycline and citric acid were added to the culture of phytoplankton, and one drop of stored plankton in which a marked development of yeasts was observed was inoculated to it, and then it was incubated at 20°C. The results are given in Fig. 4. After five

days 8×10^9 cells of yeast were detected in 1 g of the phytoplankton. In the sea near Tsuyazaki, *Doliolum* sp. (zooplankton) develops dominantly every spring (March, April). The organism was collected in April and stored in a flask at 20°C after the addition of chlortetracycline and citric acid. The number of the yeasts developing in the flask is given in Fig. 4. Since the cell of the zooplankton contains as large quantity of water as medusa does, the number of the yeasts developing in it was less than that in the decayed culture of phytoplankton. One gram of *Saccharomyces cerevisiae* consists of about 8×10^9 cells. Since the cells of most of the yeasts isolated from plankton were smaller than those of *S. cerevisiae*, 1 g of the yeasts in plankton may consists of more than 1×10^{10} cells. Consequently, the weight of 8×10^9 cells of yeast (developing in the decayed culture of phytoplankton) may be more than 0.5 g. Thus it appears that more than 50 % of cell constituents of the cultured phytoplankton were changed into the yeast cells. Diatom contains considerable amount of pectin and silicate, and it is either difficult or impossible for a microorganism to decompose these compounds. Therefore, nutrients for microorganisms in the phytoplankton (diatom) may be completely changed into the yeast cells. This idea is supported by the fact that, as is given in Plate 7, the stored plankton is completely surrounded by the pseudomycelium of the yeasts after 12 days storage as if the plankton were entirely changed into the yeast cells.

Eighty cultures of yeasts were isolated from the stored plankton and their taxonomic positions are given in Table 1.

Table 1. Yeasts collected from rotted marine plankton.

Taxon	Number of isolates
Rhodotorula flava	4
Rh. minuta	1
Rh. mucilaginosa	7
Rh. glutinis	4
Cryptococcus albidus	5
Cr. diffluens	1
Cr. laurentii	5
Cr. luteolus	1
Cr. neoformans	1
Candida tropicalis	8
C. solani	1
C. parapsilosis var. *intermedia*	9
C. brumptii	2
C. sp.	14
Trichosporon cutaneum	2
Torulopsis inconspicua	1
Black yeast	7
yeast-like fungi	7

During the first two days storage of plankton, the yeasts developing in it were *Rhodotorula* (90 %) and *Cryptococcus* (10 %). Almost all of the yeasts belonged to one or the other of these genera and a small number of Black yeasts and yeast-like fungi were present at the same time. After four to five days, *Rhodotorula* decreased to 50 % and *Cryptococcus* to less than 5 %. After seven days, when the number of the yeasts was maximum, *Candida* predominated (90 %) and the rest was *Rhodotorula*. The species of *Candida* varied according to the seasons. In summer they were *C. parapsilosis* var. *intermedia*, *C. tropicalis* and *C. brumptii*, and in winter *C.* sp.

SUMMARY

1) A remarkable development of yeasts was observed in the storage of marine plankton that was stored for several days at 20°C after the addition of chlortetracycline and citric acid. The plankton was completely surrounded by the pseudomycelium of the yeasts after 12 days storage as if the plankton were entirely changed into the yeast cells.

2) Eighty cultures of yeasts were isolated from the stored plankton. Sixteen of the cultures were members of genus *Rhodotorula*, 13 of *Cryptococcus*, 34 of *Candida*, 2 of *Trichosporon*, 1 of *Torulopsis*, 7 of Black yeast and 7 of yeast-like fungi.

3) After five days storage, 8×10^9 cells of yeasts developed in 1 g of pure cultured phytoplankton, treated with chlortetracycline and citric acid.

4) After six days storage, 2×10^9 cells of yeasts developed in 1 g of *Doliolum* sp. (zooplankton) treated with these compounds.

5) In the storage of the treated plankton, developing yeasts were *Rhodotorula* (90 %) and *Cryptococcus* (10 %) during the first two days, and after seven days *Candida* was dominant genus.

6) The species of *Candida* varied according to the seasons. In summer they were *C. parapsilosis* var. *intermedia*, *C. tropicalis* and *C. brumptii*, and in winter *C.* sp.

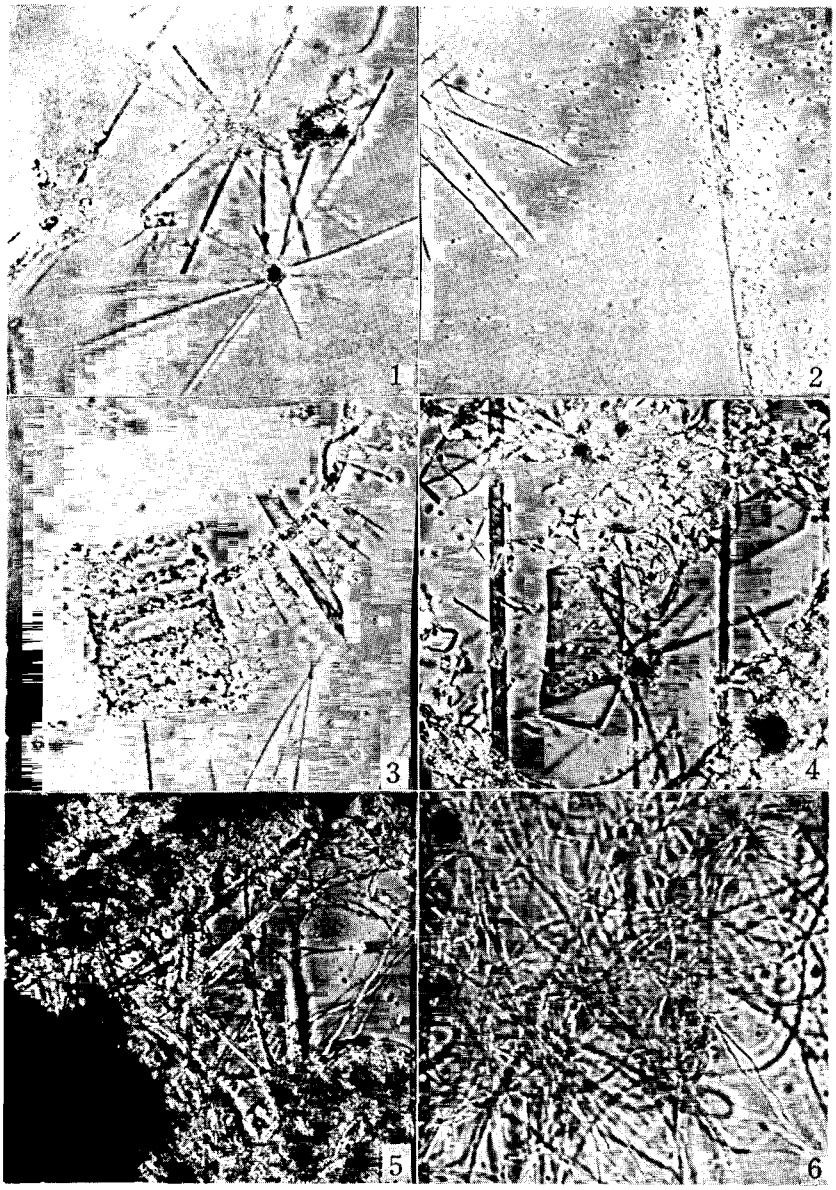
REFERENCES

1) Bhat, J. V. and N. Kachwalla, 1955. Proc. Indian Acad. Sci., Sec. B, **41**: 95.
2) ———, ———, and B. N. Moody, 1955. J. Sci. Ind. Res., Sec. C, **14**: 24.
3) Fell, J. W., D. G. Ahearn, S. P. Meyers and F. J. Roth, Jr., 1960. Limnol. and Oceanogr., **5**: 366.
4) Suehiro, S., 1960. Sci. Bull. Fac. Agr. Kyushu Univ., **17**: 449.
5) ———, 1962. Sci. Bull. Fac. Agr. Kyushu Univ. **20**: 101.
6) Yone, S., S. Fuzino and T. Tomiyama, 1960. Bull. Jap. Soc. Sci. Fish., **26**: 514.
7) Lodder, J. and N. J. W. Kreger van-Rij, 1952. The yeasts. A taxonomic study.
8) Matsue, Y., 1954. Review of Fisheries Science in Japan (in Japanese).

Explanation of Plate 7

The plate shows the appearance of the decomposition of plankton and the growth of yeasts when the plankton were stored at 20°C after addition of chlortetracycline and citric acid.

Fig. 1. After 1 days storage.
Fig. 2. After 3 days storage.
Fig. 3. After 5 days storage.
Fig. 4. After 7 days storage.
Fig. 5. After 10 days storage.
Fig. 6. After 12 days storage.



Studies on the marine yeasts. IV