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Studies on the marine yeasts. VI
On some physiological properties of the isolates

Sumio SUEHIRO* and Yukio TOMIYASU†

The physiological properties of 138 cultures of yeast, isolated from marine muds,[3] marine plankton,"? [4] and seaweeds,[1,5] were investigated : namely, the salt tolerance, the temperature range for the growth, the effect of the addition of 3 % NaCl on the growth, and the growth in peptone sea water.

The salt tolerance

The salt tolerance test was made with 3 % Malt Extract (Difco) containing NaCl ranging in concentration from 5 to 20 %. The incubation period ranged from 10 days (5, 10 %) to 20 days (15, 20 %) at 20°C.

Three cultures were able to grow in the medium containing 20 % NaCl and eight in 15 %. All of them belonged to *Torulopsis famata* or *Candida parapsilosis* var. ***intermedia.*** It is interesting that all these cultures showing high salt tolerance were isolated from seaweeds only, while the cultures of *C. parapsilosis* var. *intermedia* isolated from plankton never showed such high tolerance. All of them, moreover, have maintained the high tolerance after having been cultured on wort agar without NaCl for ten years. Since the salt concentration of the surface of seaweeds becomes higher in low tide due to evaporation, the yeasts, attaching themselves to the surface, may get such a high degree of tolerance. It is considered, therefore, that the yeasts must always attach themselves to the seaweeds in order to get such high tolerance. Moreover, the other physiological properties of the marine yeasts appear to vary according to the places where they exist.

The temperature range for the growth

The temperature range for the growth was determined by using

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3 % Malt Extract. The incubation period ranged from 2-5 days (above 20°C) to 20 days (5°C).

Among 11 cultures of C. *tropicalis* tested, four, isolated in summer, could grow at 41°C, while all of them isolated in winter could not grow even at 38°C, and at 5°C most of them isolated in summer were unable to grow or grew poorly. There were no cultures that could grow at 43°C. From the point of view of the temperature range for the growth, therefore, yeast biota in the sea seems to vary according to seasonal changes.

The effect of the addition of 3 % NaCl

The effect was tested with Hayduck's media, one containing 3 % NaCl and the other containing no NaCl, at 20°C for five days. The degree of the growth was determined with the turbidity by using photoelectric colorimeter, and the effect was determined by estimating the ratio of the growth in the medium containing 3 % NaCl to that in the medium containing no NaCl.

Most of the cultures grew better in the medium without NaCl than in that containing 3 % NaCl. Within the limit of this experiment,

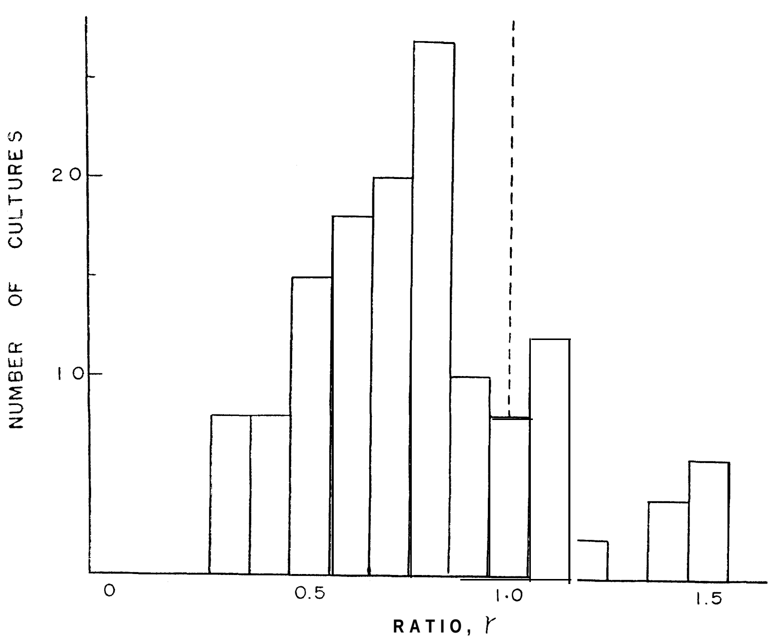

Fig. 1. Frequency distribution of the ratio, *r*,* with all the cultures tested.
**r* is obtained as the value of the ratio of the growth in the medium containing 3 % NaCl to that without NaCl.

it seems that the salt requirement of these cultures are not remarkable. However, the ratio, above mentioned, varied with cultures. This is the reason why this test was carried out. The frequency distribution of the ratio with all the cultures is given in Fig. 1. The maximal number of the cultures was observed at 0.8 (value of the ratio), the distribution itself being definable with genus. The distributions with *Candida, Cryptococcus,* and *Rhodotorula* are shown in Fig. 2.

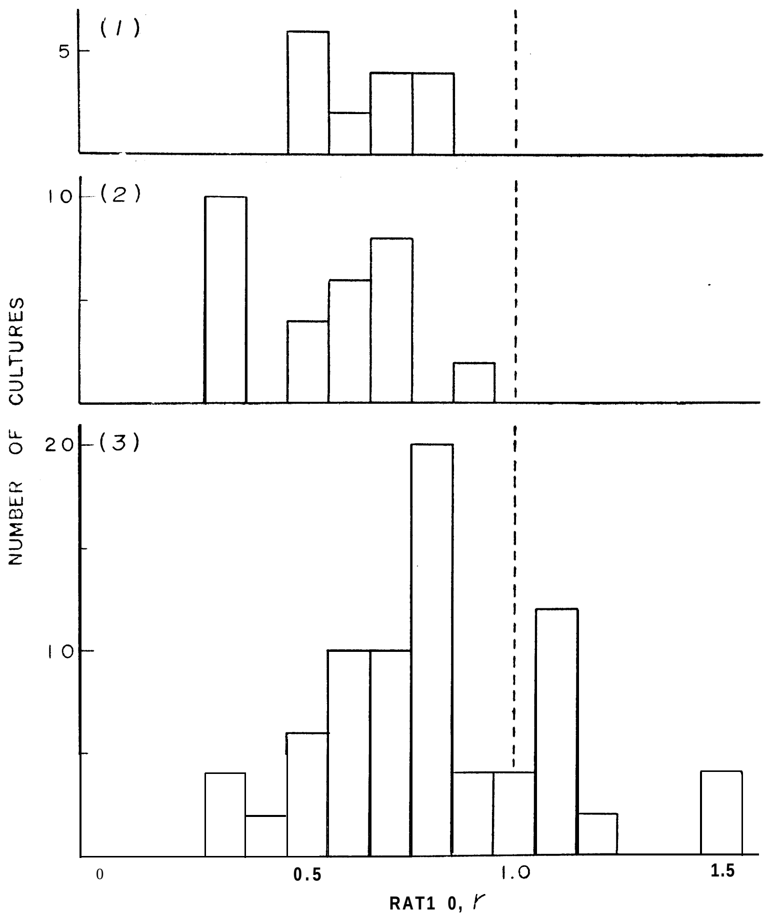

Fig. 2. Frequency distribution of the ratio, *r*, with the cultures of *Cryptococcus, Rhodotorula,* and *Candida.*
(1): *Cryptococcus,* (2) : *Rhodotorula,* (3) : *Candida*

Some cultures (especially *Torulopsis*) grew better in the medium containing 3 % NaCl than in that without NaCl. The cultures of *Candida* seemed to be more halophylic than those of *Cryptococcus* and

Rhodotorula. Though the cultures of *Torulopsis* were not enough in number to be discussed adequately, they seemed to be most halophylic among the cultures tested, and the peak of the distribution appeared to be at 1.0. From the view point of salt requirement, there seemed to be few indigenous marine species among the cultures. It is a well-known fact that most marine bacteria fail to grow in the medium without 3 % NaCl, but in our studies such cultures of yeast as fail to grow without NaCl have not been isolated. Even those cultures, showing high salt tolerance as previously mentioned, could grow well in the medium without NaCl. Among the cultures of *Candida*, some were found to be unable to produce pseudomycelium in the medium without NaCl. On counting the number of the yeasts in marine samples by plating, the ratio of the number of yeast colonies developing in the medium without NaCl to that developing in the medium containing 3 % NaCl was always found to be 1 : 1 in our experiments. In the case of marine bacteria, however, the ratio was 8 : 100.[6)] Consequently, the characteristics of indigenous marine species of yeast appear to differ considerably from that of bacteria, and in spite of lacking in strict salt requirement, following cultures may be considered as indigenous marine species :

1) The cultures unable to produce pseudomycelium in the medium not containing 3 % NaCl.

2) The cultures showing high salt tolerance.

3) The cultures growing better in the medium containing 3 % NaCl than in that without NaCl.

The growth in peptone sea water

The growth in peptone sea water was determined by using the sea water containing 0.5 % Polypeptone (Wakō Co.) at 20°C for five days. The pH of the medium was adjusted to 7.5 with NaOH.

In spite of poor sugar content in plankton, the marine yeasts utilize it well. These yeasts, therefore, may grow well with amino acids as sole source of carbon. Then, if these cultures are indigenous marine species, they may grow in the medium. As shown in Table 3, a considerable number of the cultures were able to grow well. Most of the cultures of ***Rhodotorula mucilaginosa*** grew well, while all the cultures of the other species of the genus were unable to grow or grew poorly. The characteristic of the cultures of the species seems to have some connection with the abundance of the isolates of the species from decaying plankton and seaweeds.

SUMMARY

1) Among 138 cultures of yeast tested, three cultures were able to grow in the medium containing 20 % NaCl and eight in 15 %. They belonged to *Torulopsis famata* or *Candida parapsilosis* var. *intermedia* and were isolated from seaweeds only. Such high salt tolerance was never observed on the cultures isolated from plankton. All cultures could grow in the medium containing 5 % NaCl.

2) Most of the cultures grew well at 5-30°C. Four cultures of *Candida tropicalis* isolated in summer were able to grow at 41°C, but all cultures of the species isolated in winter were unable to grow even at 38°C.

3) Most cultures grew better in the medium not containing NaCl than in that containing 3 % NaCl. The cultures of *Torulopsis* and *Candida* seemed to be more halophylic than those of *Cryptococcus* and *Rhodotorula*.

4) A considerable number of the cultures could grow well in peptone sea water (pH 7.5). Most cultures of *Rhodotorula mucilaginosa* grew well in it, but the cultures of the other species of the genus could not grow or grew poorly in it.

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6) ZoBell, C. E., 1941. J. Marine Research, 4: 42.

Table 1. The salt tolerance of the isolates.

Code	Taxon	Source	Growth at various concentration of NaCl			
			5	10	15	20
No. 1	*Saccharomyces exiguus*	marine mud	+++	—	—	—
No. 14	*S. cerevisiae*		+++	—	—	—
No. 16	*S. delbrueckii*		+++	+	—	—
No. 10	*Hansenula anomala*		+++	+++	—	—
No. 24	*Candida parapsilosis* var. *intermedia*	seaweed	+++	+	—	—
No. 164			+++	+++	—	—
No. 165			+++	+++	—	—
No. 169			+++	++	—	—
No. 170			+++	++	—	—
No. 177			+++	+++	—	—
No. 181			+++	+	—	—
No. 185			+++	++	—	—
No. 187			+++	+	—	—
No. 192			+++	+++	—	—
No. 193			+++	++	—	—
No. 194			+++	+++	+++	++
No. 197			+++	+++	—	—
No. 200			+++	++	—	—
No. 203			+++	+++	+++	++
No. 204			+++	+++	++	—
No. 84		marine plankton	+++	++	—	—
No. 98			+++	+	—	—
No. 100			++	++	—	—
No. 112			+++	++	—	—
No. 114			+++	++	—	—
No. 115			+++	++	—	—
No. 163			+++	++	—	—
No. 167			+++	++	—	—
No. 62		*T. subtilis**	+++	++	—	—
No. 63			+++	++	—	—
No. 69			+++	++	—	—
No. 70			+++	+	—	—
No. 71			+++	++	—	—
No. 31	*C. tropicalis*	marine mud	+++	+	—	—
No. 106		marine plankton	+++	+	—	—
No. 123			+++	+	—	—
No. 124			+++	+	—	—
No. 131			+++	+	—	—
No. 132			+++	—	—	—
No. 135			+++	++	—	—
No. 139			+++	++	—	—
No. 158			+++	++	—	—
No. 172			+++	++	—	—
No. 182			+++	++	—	—
No. 109	*C.* sp.		+++	+++	—	—

+ little growth, ++ moderate growth, +++ heavy growth.

* *Thalassiosira subtilis* (marine diatom)[2]

Table 1. Continued.

Code	Taxon	Source	Growth at various concentration of Nacl			
			5	10	15	20
No. 110			+++	++	—	—
No. 122			+++	++	—	—
No. 146			+++	+++	—	—
No. 147			+++	+++	—	—
No. 148			+++	+++	—	—
No. 149			+++	++	—	—
No. 150			+++	+++	—	—
No. 151			+++	++	—	—
No. 152			+++	++	—	—
No. 153			+++	—	—	—
No. 154			+++	++	—	—
No. 159			+++	++	—	—
No. 160			+++	++	—	—
No. 2	*C. brumptii*	marine mud	+++	+	—	—
No. 99		marine plankton	+++	++	—	—
No. 117			+++	++	—	—
No. 82	*C. solani*	marine plankton	+++	+++	—	—
No. 17	*C. mycoderma*	marine mud	+++	—	—	—
No. 29	*C. albicans*	seaweed	+++	—	—	—
No. 34	*C. guilliermondii*	marine mud	+++	++	—	—
No. 7	*C. intermedia*	marine mud	+++	+	—	—
No. 66	*C. lipolytica*	*T. subtilis*	+++	++	—	—
No. 67			+++	++	—	—
No. 68			+++	++	—	—
No. 36	*Rhodotorula mucilaginosa*	marine mud	+++	—	—	—
No. 38			+++	—	—	—
No. 61		*T. subtilis*	+++	—	—	—
No. 78		marine plankton	+++	—	—	—
No. 80			+++	—	—	—
No. 89			+++	—	—	—
No. 127			+++	—	—	—
No. 128			+++	—	—	—
No. 136			+++	++	—	—
No. 161			+++	++	—	—
No. 171		seaweed	+++	+	—	—
No. 174			+++	—	—	—
No. 179			+++	—	—	—
No. 188			+++	—	—	—
No. 190			+++	—	—	—
No. 195			+++	+	—	—
No. 201			+++	—	—	—
No. 104	*Rh. minuta*	marine plankton	+++	—	—	—
No. 88	*Rh. flava*	marine plankton	+++	—	—	—
No. 94			+++	—	—	—
No. 95			+++	—	—	—
No. 77	*Rh. glutinis*	marine plankton	+++	—	—	—
No. 85			+++	—	—	—

Table 1. Continued.

Code	Taxon	Source	Growth at various concentration of NaCl 5	10	15	20
No. 162			+++	++	—	—
No. 35	*Cryptococcus laurentii*	marine mud	+++	+	—	—
No. 64		*T. subtilis*	+++	—	—	—
No. 65			+++	+	—	—
No. 72			+++	+	—	—
No. 79		marine plankton	+++	++	—	—
No. 83			+++	+	—	—
No. 92			+++	—	—	—
No. 111			+++	—	—	—
No. 143			+++	++	—	—
No. 176		seaweed	+++	—	—	—
No. 178			+++	+	—	—
No. 108	*Cr. albidus*	marine plankton	+++	—	—	—
No. 119			+++	—	—	—
No. 120			+++	—	—	—
No. 125			+++	—	—	—
No. 133		marine plankton	+++	—	—	—
No. 183		seaweed	+++	—	—	—
No. 86	*Cr. diffluens*	marine plankton	+++	—	—	—
No. 175		seaweed	+++	—	—	—
No. 93	*Cr. neoformans*	marine plankton	+++	—	—	—
No. 191		seaweed	+++	+	—	—
No. 101	*Cr. luteolus*	marine plankton	+++	—	—	—
No. 156			+++	—	—	—
No. 5	*Torulopsis famata*	seaweed	+++	+++	+++	—
No. 168			+++	+++	++	—
No. 180			+++	+++	+++	—
No. 186			+++	+++	+++	++
No. 184			+++	+++	—	—
No. 196			+++	+++	—	—
No. 199			+++	—	—	—
No. 202			+++	+++	+++	—
No. 74	*T. inconspicua*	*T. subtilis*	+++	—	—	—
No. 75			+++	—	—	—
No. 76			+++	—	—	—
No. 116		marine plankton	+++	+	—	—
No. 20	*Trichosporon behrendii*	seaweed	+++	+	—	—
No. 27	*Tr. cutaneum*	seaweed	+++	+	—	—
No. 129		marine plankton	+++	—	—	—
No. 130			+++	—	—	—
No. 189		seaweed	+++	+	—	—
No. 18	*Tr. infestans*	seaweed	+++	++	—	—
No. 173			+++	++	—	—
No. 22	*Tr. pullulans*	marine mud	+++	+	—	—
No. 155	Black yeast	marine plankton	+++	+++	—	—
No. 144	yeast-like fungi	marine plankton	+++	+	—	—

Table 2. The temperature range for the growth.

Code	Taxon	Growth at various temperature					
		5	25	30	35	38	41
No. 1	*Saccharomyces exiguus*	++	+++	+++	—	—	—
No. 14	*S. cerevisiae*	++	+++	+++	—	—	—
No. 16	*S. delbrueckii*	++	+++	+++	—	—	—
No. 10	*Hansenula anomala*	++	+++	+++	—	—	—
No. 24	*Candida parapsilosis* var. *intermedia*]	++	+++	++	—	—	—
No. 164		+++	+++	++	—	—	—
No. 165		+++	+++	+++	—	—	—
No. 169		+++	+++	+++	—	—	—
No. 170		+++	+++	+++	+++	—	—
No. 177		+++	+++	+++	+++	—	—
No. 181		+++	+++	+++	—	—	—
No. 185		+++	+++	+++	+++	—	—
No. 187		+++	+++	+++	++	—	—
No. 192		+++	+++	+++	—	—	—
No. 193		+++	+++	+++	—	—	—
No. 194		+++	+++	+++	—	—	—
No. 197		+++	+++	—	—	—	—
No. 200		+++	+++	++	—	—	—
No. 203		+++	+++	+++	—	—	—
No. 204		+++	+++	+++	+++	++	—
No. 84		+++	+++	+++	—	—	—
No. 98		+++	+++	+++	+	—	—
No. 100		—	+++	+++	++	—	—
No. 112		+++	+++	+++	+++	++	—
No. 114		+++	+++	+++	+++	++	—
No. 115		+++	+++	+++	++	—	—
No. 163		+++	+++	+++	++	—	—
No. 167		+++	+++	+++	++	—	—
No. 62		++	+++	++	—	—	—
No. 63		++	+++	+++	—	—	—
No. 69		+++	+++	+++	—	—	—
No. 70		+++	+++	+++	—	—	—
No. 71		+++	+++	++	—	—	—
No. 31	*C. tropicalis*	++	+++	+++	++	—	—
No. 106		+	+++	+++	+++	—	—
No. 123		+	+++	+++	+++	+++	+++
No. 124		+	+++	+++	+++	+++	+++
No. 131		+	+++	+++	+++	+++	—
No. 132		+++	+++	+++	+	—	—
No. 135		+	+++	+++	+++	+++	+++
No. 139		+++	+++	+++	+++	+	—
No. 158		+	+++	+++	+++	+++	++
No. 172		+++	+++	+++	++	—	—
No. 182		+++	+++	+++	++	—	—
No. 109	*C.* sp.	+++	+++	+++	—	—	—
No. 110		+++	+++	++	—	—	—
No. 122		+++	+++	+	—	—	—

Table 2. Continued.

Code	Taxon	Growth at various temperature					
		5	25	30	35	38	41
No. 146		+++	+++	+++	—	—	—
No. 147		+++	+++	—	—	—	—
No. 148		+++	+++	++	—	—	—
No. 149		+++	+++	+++	+	—	—
No. 150		+++	+++	+	—	—	—
No. 151		+++	+++	++	—	—	—
No. 152		+++	+++	+	—	—	—
No. 153		+++	+++	+++	+	—	—
No. 154		+++	+++	+	—	—	—
No. 159		+++	+++	+++	+++	—	—
No. 160		+++	+++	+++	+++	++	—
No. 99	*C. brumptii*	+	+++	+++	—	—	—
No. 117		+	+++	++	—	—	—
No. 2		+	+++	+++	—	—	—
No. 82	*C. solani*	+	+++	+++	+++	—	—
No. 17	*C. mycoderma*	++	+++	++	—	—	—
No. 29	*C. albicans*	—	+++	+++	+++	+++	—
No. 34	*C. guilliermondii*	—	+++	+++	+++	+++	—
No. 7	*C. intermedia*	++	+++	+++	+++	—	—
No. 66	*C. lipolytica*	++	+++	+++	—	—	—
No. 67		++	+++	+++	—	—	—
No. 68		++	+++	+++	—	—	—
No. 36	*Rhodotorula mucilaginosa*	++	+++	—	—	—	—
No. 38		++	+++	+	—	—	—
No. 61		++	+++	+	—	—	—
No. 78		+++	+++	—	—	—	—
No. 80		+++	+++	—	—	—	—
No. 89		+++	+++	+	—	—	—
No. 127		+++	+++	+++	—	—	—
No. 128		+++	+++	+++	—	—	—
No. 136		+++	+++	+++	—	—	—
No. 161		+++	+++	—	—	—	—
No. 171		+++	+++	+	—	—	—
No. 174		+++	+++	—	—	—	—
No. 179		+++	+++	+	—	—	—
No. 188		+++	+++	—	—	—	—
No. 190		++	+++	+	—	—	—
No. 195		++	+++	+	—	—	—
No. 201		++	+++	+	—	—	—
No. 104	*Rh. minuta*	+	+++	++	—	—	—
No. 88	*Rh. flava*	+++	+++	++	—	—	—
No. 94		+++	+++	++	—	—	—
No. 95		+++	+++	++	—	—	—
No. 77	*Rh. glutinis*	+++	+++	++	—	—	—
No. 85		+++	+++	++	—	—	—
No. 162		+++	+++	—	—	—	—
No. 35	*Cryptococcus laurentii*	++	+++	—	—	—	—

Table 2. Continued.

Code	Taxon	Growth at various temperature					
		5	25	30	35	38	41
No. 64		+++	+++	—	—	—	—
No. 65		+++	+++	—	—	—	—
No. 72		+++	+++	—	—	—	—
No. 79		+++	+++	—	—	—	—
No. 83		+++	+++	—	—	—	—
No. 92		+++	+++	—	—	—	—
No. 111		+++	+++	—	—	—	—
No. 143		—	+++	+++	—	—	—
No. 176		+++	+++	—	—	—	—
No. 178		+++	+++	—	—	—	—
No. 108	*Cr. albidus*	++	+++	++	—	—	—
No. 119		++	+++	++	—	—	—
No. 120		+++	+++	+	—	—	—
No. 125		+++	+++	++	—	—	—
No. 133		++	+++	++	—	—	—
No. 183		++	+++	++	—	—	—
No. 86	*Cr. diffluens*	++	+++	++	—	—	—
No. 175		++	+++	++	—	—	—
No. 93	*Cr. neoformans*	++	+++	++	—	—	—
No. 191		++	+++	++	—	—	—
No. 101	*Cr. luteolus*	—	+++	++	—	—	—
No. 156		—	+++	++	—	—	—
No. 5	*Torulopsis famata*	++	+++	+++	—	—	—
No. 168		+++	+++	+++	—	—	—
No. 180		+++	+++	+++	—	—	—
No. 184		+++	+++	+++	—	—	—
No. 186		+++	+++	+++	—	—	—
No. 196		+++	+++	+++	++	—	—
No. 199		+++	+++	+++	++	—	—
No. 202		—	+++	+++	+	—	—
No. 74	*T. inconspicua*	—	+++	+++	+++	++	—
No. 75		—	+++	+++	+++	++	—
No. 76		—	+++	+++	+++	++	—
No. 116		—	+++	+++	+++	++	—
No. 20	*Trichosporon behrendii*	—	+++	+++	++	—	—
No. 27	*Tr. cutaneum*	++	+++	+++	—	—	—
No. 129		++	+++	+++	+++	+	—
No. 130		++	+++	+++	+++	—	—
No. 189		++	+++	+++	+++	—	—
No. 18	*Tr. infestans*	+	+++	+++	—	—	—
No. 173		—	+++	+++	—	—	—
No. 22	*Tr. pullulans*	+++	++	—	—	—	—
No. 155	Black yeast	+	+++	+++	++	—	—
No. 144	yeast-like fungi	+	+++	+++	++	—	—

Table 3. The growth in peptone sea water.

Code	Taxon	Growth	Code	Taxon	Growth
No. 1	*Saccharomyces exiguus*	—	No. 139		⧻
No. 14	*S. cerevisiae*	—	No. 158		+
No. 16	*S. delbrueckii*	—	No. 172		+
No. 10	*Hansenula anomala*	+	No. 182		⧺
No. 24	*Candida parapsilosis* var. *intermedia*	+	No. 109	*C.* sp.	+
No. 164		⧺	No. 110		+
No. 165		⧺	No. 122		+
No. 169		+	No. 146		⧺
No. 170		+	No. 147		⧺
No. 177		—	No. 148		⧻
No. 181		—	No. 149		+
No. 185		+	No. 150		⧺
No. 187		—	No. 151		+
No. 192		—	No. 152		⧺
No. 193		+	No. 153		+
No. 194		⧻	No. 154		⧻
No. 197		—	No. 159		+
No. 200		—	No. 160		+
No. 203		⧺	No. 2	*C. brumptii*	—
No. 204		⧺	No. 99		⧻
No. 84		⧺	No. 117		⧻
No. 98		+	No. 82	*C. solani*	—
No. 100		—	No. 17	*C. mycoderma*	—
No. 112		—	No. 29	*C. albicans*	⧻
No. 114		—	No. 34	*C. guilliermondii*	+
No. 115		—	No. 7	*C. intermedia*	⧺
No. 163		—	No. 66	*C. lipolytica*	⧻
No. 167		+	No. 67		⧻
No. 62		+	No. 68		⧻
No. 63		—	No. 36	*Rhodotorula mucilaginosa*	—
No. 69		⧺	No. 38		—
No. 70		+	No. 61		—
No. 71		—	No. 78		+
No. 31	*C. tropicalis*	⧺	No. 80		—
No. 106		+	No. 89		+
No. 123		⧺	No. 127		+
No. 124		⧺	No. 128		⧻
No. 131		+	No. 136		⧻
No. 132		⧺	No. 161		⧻
No. 135		⧻	No. 171		⧻

Table 3. Continued.

Code	Taxon	Growth	Code	Taxon	Growth
No. 174		+++	No. 186		+
No. 179		+++	No. 196		—
No. 188		+++	No. 199		—
No. 190		+++	No. 202		+
No. 195		+	No. 74	*T. inconspicua*	—
No. 201		—	No. 75		—
No. 104	*Rh. minuta*	+	No. 76		—
No. 88	*Rh. flava*	—	No. 116		+
No. 94		—	No. 20	*Trichosporon behrendii*	—
No. 95		+	No. 27	*Tr. cutaneum*	++
No. 77	*Rh. glutinis*	+	No. 129		+++
No. 85		—	No. 130		+++
No. 162		+	No. 189		++
No. 35	*Cryptococcus laurentii*	—	No. 18	*Tr. infestans*	+++
No. 64		+	No. 173		+++
No. 65		—	No. 22	*Tr. pullulans*	+
No. 72		—	No. 155	Black yeast	+
No. 79		—	No. 144	yeast-like fungi	+++
No. 83		+			
No. 92		—			
No. 111		—			
No. 143		+			
No. 176		+			
No. 178		+			
No. 108	*Cr. albidus*	—			
No. 119		—			
No. 120		—			
No. 125		—			
No. 133		+			
No. 183		—			
No. 86	*Cr. diffluens*	+			
No. 175		+			
No. 93	*Cr. neoformans*	—			
No. 191		+			
No. 101	*Cr. luteolus*	+			
No. 156		+			
No. 5	*Torulopsis famata*	+++			
No. 168		+			
No. 180		+			
No. 184		++			