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Fundamental Studies on Physiology of Rotifer for its Mass Culture—IV Nutritional Effect of Yeast on Population Growth of Rotifer*

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The nutritional effect of caked yeast still living and of dried marine yeast on the rotifer, *Brachionus plicatilis*, was examined.

Experiments were performed by culturing many individuals in many test tubes, each containing two individuals in the experimental medium. From daily counts of eggs laid and surviving individuals, time intervals from hatching to 50% survival and to peak of fecundity, net reproduction rate, intrinsic rate of population increase and mean generation time were estimated.

The results obtained are summarized as follows.

1. Caked yeast still living, *Rhodotorula sp.* has less nutritional effect on the rotifer than that of the marine *Chlorella*. However, it seems to be somewhat effective as food for the rotifer.

2. Dried marine yeast itself has little nutritional effect on the rotifer. However, aerobical decomposition can increase the nutritional effect to some extent.

3. The marine yeast added to a diluted *Chlorella* suspension was very effective as supplementary food for the rotifer. Hence, yeast may be used most effectively as a supplementary food in the culture medium when sufficient phytoplankton can not be supplied to the rotifer.

The mass culture of the rotifer, *Brachionus plicatilis*, 'Shiomizutsubowamushi' in Japanese is performed mainly with a marine species of *Chlorella*. However, recently, there are some attempts to use yeast as a food of the rotifer, instead of the green phytoplankton.

In the present study, therefore, caked yeast still living and dried marine yeast were tested for nutritional effect on the rotifer. Moreover, the latter was tested for the effect of aerobical decomposition on its nutritional value and for its supplementary effect on insufficient supply of the *Chlorella*.

Materials and Methods

The rotifer, *Brachionus plicatilis*, and the *Chlorella* used as a food plankton in basic culture were derived from the same clones as those in the preceding investigations on the effect of temperature[1] and density of phytoplankton.[2] The caked yeast still living, *Rhodotorula sp.* and the dried marine yeast abbreviated to ASY–4011, were produced by Kanegafuchi Kagaku Co. Ltd. for fish culture and by Asahi Kasei Co., Ltd., respectively.

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The parthenogenetic eggs offered to experiments were collected by the method described in the previous report.[1] They were the first-laid eggs just before hatching of amictic females of the group which had been actively increasing by feeding with the *Chlorella* at least for 10 days before experiment, except in the experiment on caked yeast, with which the rotifer had been fed. During pre-culture for collection of the first-laid eggs from those females, they were cultured under experimental condition by feeding with each food material. The number of individuals offered to each experiment ranged from 26 to 36. They were cultured in many test tubes each containing two individuals in experimental medium. These culture media were renewed daily and at that time the number of eggs laid and of surviving individuals in all tubes was counted. From these daily counts time intervals to 50% survival (M) and to peak of fecundity (P) from hatching were obtained and net reproduction rate (R_0), intrinsic rate (r) of population increase and mean generation time (T) at each food material were estimated on the basis of BIRCH's computational methods.[3] More details of experimental procedure and of explanation of these indices were referred to the description in the previous report.[1]

Caked yeast was made to suspend in sea water at the rate of 50 μg/ml. Then, 3 ml of the suspension was introduced into many test tubes for the investigation of nutritional effect on the rotifer.

Dried marine yeast with or without aerobical decomposition was tested for nutritional effect on the rotifer and for supplementary effect on insufficient supply of the *Chlorella* to the rotifer. It was filtered through the sieve with 175 mesh and made to suspend in sea water at the rate of 220 μg/ml. Then, the suspension was divided into two parts, which were autoclaved (120°C, 1kg/cm^2, 20 min.). One part was allowed to stand at least for 10 days under sterilized condition to be in equilibrium with air, and the other was exposed to raw air during the same period to be aerobically decomposed. During decomposition, a few protozoa could be observed in the suspension, but no phytoplankton at all. The former part was used for the experiment on the effect of the marine yeast itself, and the latter was used for the experiment of its aerobically decomposed product. In both experiments, mixture of 0.5 ml of each part and 2 ml of sterilized sea water in equilibrium with air was introduced as food suspension into test tubes. Therefore, experimental medium contained the marine yeast at the rate of 44 μg/ml. Both experiments were performed in parallel with individuals divided from a group of first-laid eggs.

In order to examine supplementary effect of the marine yeast, experiments were performed with two mixed suspensions (4 ml) in which the sterilized yeast was added to the *Chlorella* suspension (21×10^4 cells/ml) at the rates of 25 μg/ml and 50 μg/ml, respectively. Two experiments were also performed in parallel. The results were compared with that of the experiment on the *Chlorella* suspension without yeast. In this

experiment the rotifer was precultured with the *Chlorella* until collection of first-laid eggs, which were washed thoroughly several times with sterilized sea water, though they could not be completely free of bacteria. All tools were also sterilized before use.

Culture medium used in the investigation was prepared with the sea water treated with charcoal, and it was at Cl, temperature and pH of about 12.8‰., 22°C±0.1°C, and about 7.5, respectively.

Results

Shown in Fig. 1 are the survivorship and fecundity curves obtained from various experiments. Values of various indices concerning population growth of the rotifer are shown in Table 1.

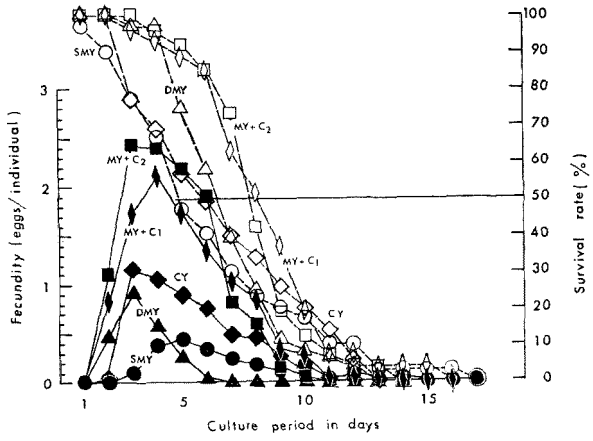

Fig. 1. Survivorship and Fecundity of the rotifer cultured only with caked yeast, only with marine yeast and with both food materials of marine yeast and *Chlorella*.

Fecundity	Survival rate	
—◆—	—◇—	Caked yeast (50 μg/m*l*)
—●—	—○—	Sterilized marine yeast (44 μg/m*l*)
—▲—	—△—	Decomposed marine yeast (44 μg/m*l*)
—◆—	—◇—	Marine yeast (25 μg/m*l*)+*Chlorella* (21×10^4 cells/m*l*)
—■—	—□—	Marine yeast (50 μg/m*l*)+*Chlorella* (21×10^4 cells/m*l*)

The group of the rotifer fed with the caked yeast is less survival than the other groups except for the one fed only with sterilized marine yeast, as shown by time (M) to 50% survival from start of experiment. The net reproduction rate (Ro) and the intrinsic rate (r) of population increase show slightly low values of 5.5 and 0.36, respectively, in comparison with those of the rotifer's groups fed with the *Chlorella*. However, they are much higher than those of the groups fed with the dried marine yeast treated in two different ways.

Table 1. The values of various indices concerning population growth of the rotifer cultured only with caked yeast still living, only with marin yeast, only with *Chlorella* and with both food materials of marine yeast and *Chlorella*.

Index	Caked yeast (50 μg/ml)	Sterilized marine yeast (44 μg/ml)	Decomposed marine yeast (44 μg/ml)	Marine yeast (25 μg/ml)+ *Chlorella* (21×10^4 cells/ml)	Marine yeast (50 μg/ml)+ *Chlorella* (21×10^4 cells/ml)	*Chlorella* (21×10^4 cells/ml)
M	5.9	4.9	6.4	8.1	7.7	10.5
P	3	5	3	4	3	3
T	4.7	6.1	3.2	4.2	3.8	5.0
R_0	5.5	2.1	2.2	10.3	11.6	7.7
r	0.36	0.12	0.25	0.56	0.64	0.41

M: days to 50% survival from start of experiment
P: days to peak of fecundity from start of experiment
T: mean generation time
R_0: net reproduction rate
r: intrinsic rate of population increase

Both groups of the rotifer fed only with the marine yeast treated in two different ways are much less survivable than the ones fed with the *Chlorella*, as shown by time (M) to 50% survival from start of experiment. Between the rotifer's groups fed only with the marine yeast, a group with the sterilized marine yeast is less survivable than that with aerobically decomposed product. As shown in Fig. 1, the rotifer in the former group has a tendency to lay eggs little by little throughout her life, while the rotifer in the latter group lays eggs in short duration of the early part of her life. This can be known from the difference between the two groups in time (P) to peak of fecundity from start of experiment. Nevertheless, net reproduction rates (R_0) of the rotifer's groups fed in two ways show almost the same low values; 2.06 in the former case and 2.23 in the latter case. Reflecting these facts, both intrinsic rates (r) of population increase have very low values in comparison with those of the rotifer's groups fed with the *Chlorella*. However, in the case of the rotifer fed with the aerobically decomposed yeast, it has much higher value of 0.25 than the value of 0.12 in the case fed with the yeast without decomposition.

The courses of experiments* on supplementary effect of the marine yeast to the marine *Chlorella* are also shown in Fig. 1, and the values of indices are shown in Table 1 together with those of a rotifer's group cultured only with the *Chlorella* suspension at the same density (21×10^4 cells/ml). Time (M) to 50% survival from start of experiment obtained from the two rotifer's groups fed with both food materials of the marine yeast and the *Chlorella* are longer than those in the any groups of the rotifer fed only with yeast, but shorter than that in the rotifer's group fed only with the *Chlorella* at the same density. Net reproduction rates (R_0) and the intrinsic rates (r) of population increase

* At the sixth night from start of experiment, water temperature accidentally droped to about 14°C for several hours.

obtained from the first two groups are much higher than those in all groups fed only with the *Chlorella* or only with the yeast.

Discussion and Conclusion

The caked yeast still living seems to be somewhat effective as a food of the rotifer for the population growth though it is less effective than the *Chlorella*.

The dried marine yeast without decomposition has little nutritional effect* on the rotifer. But if the values of indices were obtained by actual mass culture, they might be higher than those obtained in this experiment, in which the daily handling might slightly depress the rotifer.[4]

The results clarify that yeast may be used most effectively as supplementary food in the culture medium when sufficient phytoplankton can not be supplied to the rotifer. However, there have been some cases of success in the mass culture by using only yeast.[5] This success may be due to difference in nutritional effect (nutrient constituents or digestibility) of the yeast from the ones used in this study. Moreover it can not be ignored that the decomposed product of the yeast, and bacteria and phytoplankton growing in culture medium might have resulted in the above success.

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* There remains a possibility that heating process of autoclaving might decrease essential nutrients.