Supplementary Effect of Vitamin C and Squid Liver Oil on the Nutritional Value of Baker's Yeast for the Population Growth of the Rotifer *Brachionus plicatilis*

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The present study investigated the supplementary effect of vitamin C and squid liver oil on the nutritional value of baker's yeast for the population growth of the rotifer *Brachionus plicatilis*. Rotifers were axenically cultured in the basic food suspension and in the food suspension containing the nutrients.

Vitamin C showed supplementary effect at all concentrations tested. Highest rotifer growth was observed when the vitamin was added at 4.0 ug/ml to the basic food suspension. Rotifer growth was also improved by addition of squid liver oil to the yeast suspension. This indicates that the rotifer incorporated squid liver oil as exogenous source of fatty acids for its own purpose.

Key words: rotifer, baker's yeast, nutritional value, vitamin C, squid liver oil

Introduction

In mass culture of the rotifer *Brachionus plicatilis,* suppressed growth or crash of the culture is often experienced when baker's yeast is used as food[1]. Hirayama and Funamoto[2] reported that washed cells of baker's yeast is a nutritionally deficient food for the rotifer and cannot support growth under axenic conditions. Successful mass culture with baker's yeast may be due to the supplementary effects of bacteria and phytoplanktons which propagate in the culture tanks by utilizing nutrients released during decomposition of yeast[3]. Hence, clarifying the nutritional deficiency of baker's yeast may give clues to the improvement of its nutritional value for the rotifer. It may also add to knowledge on the nutritional requirements of the rotifer.

Vitamin B_{12} was found to be an essential nutrient for the growth of the rotifer[2, 4], and its addition to the yeast suspension greatly improved the nutritional value of baker's yeast for the rotifer. The fat-soluble vitamins A, D and E also showed supplementary effect on the nutritional value of baker's yeast for the rotifer growth when added to the vitamin B_{12} enriched yeast suspension[5]. However, rotifer growths in enriched yeast suspensions were still much lower than growth obtainable by culturing with *Nannochloropsis oculata,* an excellent food for the rotifer[6]. This implies that other nutrients essential for the rotifer is still lacking in baker's yeast.

The present study investigated the supplementary effects of vitamin C and squid liver oil on the nutritional deficiency of baker's yeast for the population growth of the rotifer.

Materials and Methods

In general, methods employed for the study were the same as those described in a previous investigation[5]. First laid eggs collected from an actively growing group of rotifer were axenically cultured in the basic food suspension

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and the experimental food suspension.

The experimental food suspension contained the basic food suspension plus the nutrient under investigation. The supplementary effect of the nutrient was evaluated by comparing population growth indices obtained from rotifer cultures. Baker's yeast had been cultured in 200 ml flasks containing Mayer's medium with continuous aeration after purification by picking up its colony from an agar plate culture. Cells employed were under the exponential stage of growth and were washed prior to resuspension in the rotifer culture water. The food suspensions were shaked with a Circle Shaker (Taiyo Co. Ltd.) set at 130 rotations/min. for 15 minutes 6 times a day to keep yeast cells in a suspended state throughout the investigation[7].

The rotifer used for the experiments were amictic females derived from the same strain (L type) employed in a previous investigation[5]. During investigations, temperature was maintained at 23°C and no occurence of mictic females or males was observed. The population growth indices used for evaluation were determined from the batch culture and individual culture methods. In batch culture method, the increase in number of individuals was determined as the index, after culturing rotifers in test food suspensions for 6 days from inoculation of 20 individuals for each experimental group. In individual culture method, the intrinsic rate of population increase (r) and the net reproduction rate (R_0) were calculated from the survival rates and fecundities obtained by daily observation with the renewal of food suspensions during the whole lifespan. The two culture methods are described in details elsewhere[8].

The supplementary effect of vitamin C on the nutritional deficiency of baker's yeast for the rotifer was evaluated by adding vitamin C at a concentration range of 0.4 to 8.0 μg/ml to the basic food suspension. The basic food suspension consisted of 200 μg/ml of baker's yeast and enriched with vitamins B_{12}, A, D and E at 1.4, 2.0, 0.2 and 1.0 μg/ml respectively. Above-mentioned vitamins were added to the basic food suspensions in order to allow growth of rotifer even in the control groups. The concentrations of vitamins added were those which showed the highest supplementary effects in the previous investigation[5].

Evaluation of the effect of squid liver oil at a concentration range of 2.0 to 8.0 μg/ml was conducted by individual culture method. A comparison between the nutritional quality of squid liver oil enriched food suspension and fat-soluble vitamins enriched food suspension was also investigated. Squid liver oil and fat-soluble vitamins were added to the food suspension in emulsified state. The basic food suspension consisted of 200 μg/ml of baker's yeast and 1.4 μg/ml of vitamin B_{12}.

Squid liver oil was a product of Riken Vitamin Co. Ltd. Procedures for the collection of first laid eggs, sterilization using antibiotic mixtures, preparation of food suspensions including emulsification of fat-soluble nutrients and sterility tests using STP medium are described elsewhere[2, 5].

Results and Discussion

The effect of vitamin C at varying concentrations in the yeast suspension on the rotifer growth when evaluated using individual culture method is as shown in Table 1. Fig. 1 shows the indices expressed as relative values by dividing data (Table 1) obtained from experimental food suspensions by those obtained from their respective control groups. As shown in Fig. 1, all rotifer groups cultured in suspensions containing vitamin C showed relative indices greater than 1. Highest relative indices were observed at a concentration of 4.0 ug/ml. A similar trend was observed in batch cultures (Fig. 2). The highest rotifer growths after 6 days of culture was also observed when vitamin C was supplemented at a concentration

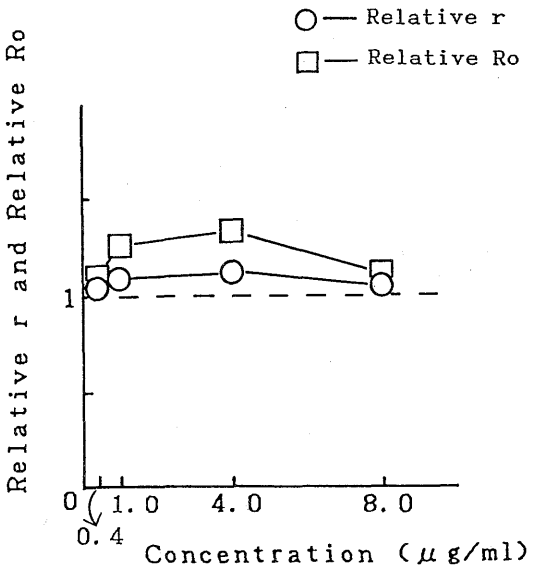

Fig. 1. Relative r and relative Ro for vitamin C at different concentrations.

Table 1. Indices r and Ro obtained from individual culture method for vitamin C

Concentration of vitamin C added in μg/ml*	Experiment 1		Experiment 2	
	r	Ro	r	Ro
0	0.4	6.28	0.38	6.37
0.4			0.40	7.08
1.0	0.43	7.93		
4.0	0.45	8.36		
8.0			0.41	7.56

*Vitamin C was added to the basic food suspension consisting of 200 μg/ml of baker's yeast and vitamins B_{12} A, D & E at 1.4, 2.0, 0.2, & 1.0 μg/ml respectively.

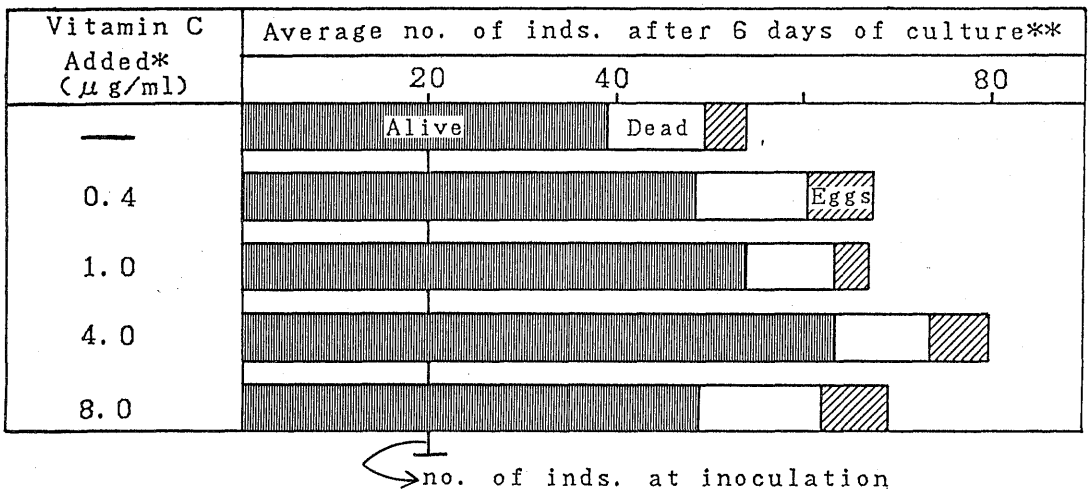

Fig. 2. Average increase in population of the rotifer in food suspensions supplemented with vitamin C at different concentrations using batch culture method.
*Vitamin C was added to basic food suspension consisting of 200 μg/ml of baker's yeast and of vitamins B_{12}, A, D and E at 1.4, 2.0, 0.2 and 1.0 μg/ml respectively.
**Average values of two replicates.

of 4.0 μg/ml.

The effect of vitamin C on the growth of the rotifer also implied that the rotifer exhibits requirement for the vitamin. The mode of vitamin C uptake of the rotifer may be similar to other water-soluble nutrients since it can also be taken directly from the solution. It was not determined however whether vitamin C shows inhibitory effect when added at higher concentrations.

The effect of squid liver oil on the growth of the rotifer evaluated from individual culture method is shown in Table 2. All groups cultured in suspensions supplemented with

Table 2. Indices r and Ro obtained from individual culture method for squid liver oil and vitamins A, D & E

Nutrients added to basic food suspension*	Concentration in μg/ml	Experiment 1 r	Ro
squid liver oil	0	0.34	4.2
	2.0	0.34	4.72
	4.0	0.51	9.57
	8.0	0.44	6.86
		Experiment 1 r	Ro
none	−	0.46	5.65
squid liver oil	4.0	0.56	10.84
vits. A, D & E	2.0, 0.2 & 1.0	0.52	7.85

*The nutrients were added to the basic food suspension consisting of 200 μg/ml of baker's yeast and 1.4 μg/ml of vitamin B_{12}.

squid liver oil showed higher growth indices than the control group. Highest growth indices were observed when supplemented at 4.0 μg/ml, wherein the r and Ro values were 0.51 and 9.57 respectively. These were 1.5 and 2.3 times higher than the respective values obtained from the control group. A comparison of the nutritional values between fat-soluble vitamins enriched suspension and squid liver oil enriched suspension showed higher growth of the rotifer in suspensions supplemented with squid liver oil (Table 2).

During mass culture of the rotifer, squid liver oil is usually offered to increase fatty acid contents of the rotifer for larvae of marine species. The present investigation indicates that the rotifer also incorporates squid liver oil as exogenous source of fatty acids for its own purpose, even though the rotifer is also reported capable of fatty acid synthesis[9]. Rotifers fed with ω-yeast, which contains high amounts of fatty acids, were also reported to be significantly bigger in size than those fed with baker's yeast[10]. These results indicate that lipids offered to the rotifer are utilized by the rotifer itself.

Although vitamin C and squid liver oil were found to improve the nutritional value of baker's yeast, growth of rotifer obtained were still much lower than that obtainable by culturing with *N. oculata*. Hence, other factors influencing the nutritional value of baker's yeast for the rotifer remains to be studied.

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ビタミンCとイカ肝油添加によるパン酵母のシオミズツボワムシに対する栄養価値の改善

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パン酵母のシオミズツボワムシ（以下ワムシと略記）に対する餌料的欠陥は各種栄養素の添加によって，改善できることはすでに報告されている。本実験では，パン酵母の栄養価値改善に関する基礎的研究の一環として，ビタミンCとイカ肝油のパン酵母に対する補強効果を調べた。基本餌料懸濁液とそれに試験栄養素を加えた試験餌料懸濁液とでL型ワムシを無菌的に飼育し，その増殖を比較することによって添加効果を判定した。

ビタミンCを各濃度段階で基本餌料懸濁液に添加した場合，基本餌料に比べてワムシは高い増殖を示した。また，イカ肝油を酵母の懸濁液に添加することによってワムシの増殖が向上したことから，ワムシがイカ肝油を体内に取り込み，かつ栄養源として利用していることも明らかになった。