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A Mass-Culture Method for *Artemia salina* Using Bacteria as Food*

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Abstract: The present experiments were carried out to clarify the relative food value of bacteria and to develop a method for the mass-culture of *Artemia* using bacteria as food. By performing screening tests on various bacteria as available food for *Artemia* larvae, the B-9 strain of *Acinetobacter* sp. was found to be effective. Larval culture using the bacterial strain and *Chlamydomonas* sp. as food for estimation of relative food value indicated that the B-9 strain food value is slightly less than that of *Chlamydomonas* sp. Several culture conditions were examined to test the feasibility of utilizing bacteria for mass-culture. The results showed that the most effective culture condition was a food mixture of the B-9 strain at a concentration of 10^8/m*l* and *Chlorella* sp. at 10^6/m*l*, with a density of *Artemia* of 5 indiv./m*l* and a culture temperature of 20°C.

1. Introduction

Nowadays, in many countries, the following live foods are being used for mariculture purposes: *Rotifer*, *Artemia*, *Daphnia*, *Moina*, *Polyphemus*, and *Gammarus* (JHINGRAN and GOPALAKRISHAN, 1974). *Artemia* is the most frequently used and the most convenient form among these organisms, as it is possible to readily obtain various sizes of larvae which can be fed to predators of different size, whenever necessary.

Several studies have attempted to clarify the nutritional preferences of *Artemia*. TERAMOTO and KINOSHITA (1961) fed the shrimp on Wakamoto***, bread yeast, and the dried residue of acetone-butanol fermentation. KUSAKABE (1964) found that wheat flour mixed with soybean powder was a very effective food for the shrimp. Furthermore, cultures of some kinds of unicellular Chlorophyceae, Chrysophyceae, Cryptophyceae, and diatoms have successfully been fed to shrimp (PROVASOLI *et al.*, 1959; GILCHRIST, 1960; NIMURA, 1963; LASKER, 1966; TAKANO, 1967). However, experiments on bacteria as food for the shrimp have been rare (SEKI, 1966).

The purpose of the present investigation was to clarify the relative food value of various strains of marine bacteria and to develop a method for the mass-culture of *Artemia* using bacteria as food. The shrimp used in the experiments was *Artemia salina* LEACH, dried eggs of which were obtained commercially through the Shintoa-Koeki Co, Ltd. from California, U.S.A. The experiments were carried out at the Tamano Marine Station, Japan Fisheries Farming Association (JFFA), and at the Marine Microbiology Laboratory, Ocean Research Institute, University of Tokyo, during the period from May to October, 1977.

2. Materials and methods

Screening of isolates as food Screening tests of potential food for *Artemia* larvae were carried out on isolates of 12 strains of bacteria and one strain of yeast. The isolates were collected from cultures of microbial flock using equipment of the Tamano Marine Station, JFFA.

The culture vessel was a 500-m*l* cylindrical beaker, containing 400 m*l* sterilized seawater,

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*** A commercial drug made of cultures of several types of commonly used molds on cereal germs, and enriched with active lactobacilli and dried yeast.

at room temperature of about 20°C, with aeration. A cylindrical net (100 μm mesh size), sterilized with 70% ethanol, was aseptically placed on the beaker. Then, 100 nauplii of *Artemia* hatched germ-free by the method of PROVASOLI *et al.* (1959) were transferred into the net. The isolates were grown to late logarithmic phase in Medium 2216E broth (OPPENHEIMER and ZOBELL, 1952), and the cultures were harvested by centrifuging at 4,000 ×g for 15 min. Packed cells were added to the culture vessel to obtain a concentration of 10^8/m*l*. After the nauplii had fed on the isolates for 4 hours, the net with the nauplii was removed and held in a beaker containing 400 m*l* sterilized seawater for 20 hours.

This was repeated every 24 hours for one week, and during the experimental period the numbers of surviving larvae were recorded.

Estimation of food value Determinations were made of the food value for *Artemia* larvae of three kinds of food, including the B-9 and P strains confirmed by the screening test as the most suitable foods, and *Chlamydomonas* sp. reported by SICK (1976) to be the most efficient food. The experimental procedure was the same as that mentioned above. Arbitrary concentrations of food were set at 5×10^5/m*l* for *Chlamydomonas* sp. and 10^8/m*l* for the two bacterial strains. 200 newly hatched nauplii were accomodated in each. The experiment was continued for 11 days, and during this period the numbers of survivors, body length and body weight of the larvae were observed.

Mass-culture of Artemia The B-9 strain (confirmed by the screening test as the most suitable food for *Artemia* larvae), the microbial flock (produced by the method of IMAMURA and SUGITA (1972)), and *Chlorella* sp. (cultured at Tamano Marine Station) were used as food for large scale larval culture.

The experiments were divided into four steps. In the first step, arbitrarily chosen culture conditions were set up as follows; culture density of the larvae was 1 indiv./m*l*, water temperature was 20°C, and food species were supplied independently at concentrations of 10^7/m*l* of *Chlorella* sp., 10^8/m*l* of the B-9 strain, and 10^8/m*l* of the microbial flock.

In the second step, to examine whether a combined microbial food was more suitable, four combinations of food concentrations were selected. The B-9 strain (10^8/m*l*) was combined with *Chlorella* sp. at 10^6/m*l* and 10^7/m*l*, and the microbial flock (10^8/m*l*) was also combined with *Chlorella* sp. at 10^6/m*l* and 10^7/m*l*. Water

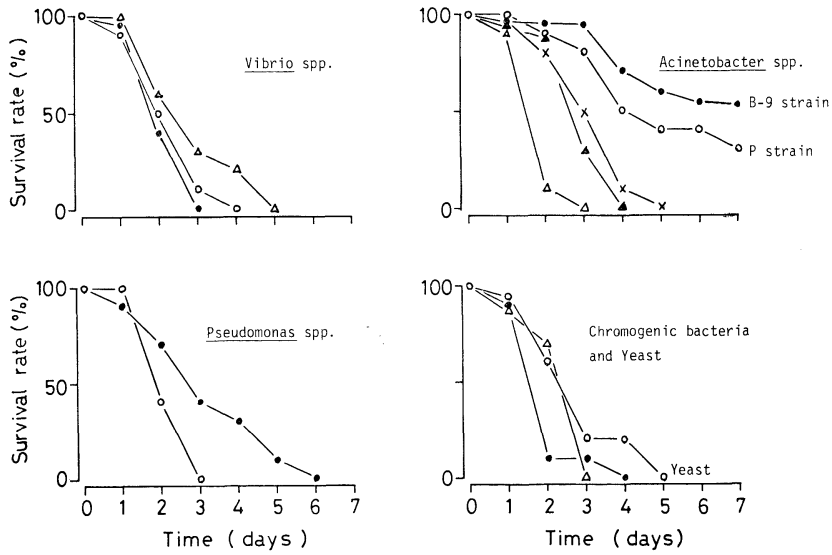

Fig. 1. Effect of different bacterial and yeast strains as food on the survival rate of *Artemia*.

temperature and larval density were 20°C and 1 indiv./m*l*, respectively.

The third step in the investigation was carried out to determine the most suitable density for mass-culture. Four kinds of larval densities were selected at 1, 5, 20 and 50 indiv./m*l*. The larvae were cultured on the mixed food of the B-9 strain (10^8/m*l*) and *Chlorella* sp. (10^6/m*l*) with a larval density of 5 indiv./m*l*, which was confirmed by the third series of experiments as the most suitable.

The culture vessel for the experimental mass-culture of *Artemia* was a 500 *l* capacity square slate tank, into which 500 *l* of sand-filtered seawater was added. A rate of aeration was 1 *l*/min., and a continuous illumination of 200 lux was provided by fluorescent light. Agitation was provided by an agitator in order to prevent precipitation of the food and the larval faecal pellets, and further to raise food value. The cultures of the B-9 srtain, the microbial flock and *Chlorella* sp. were harvested by continuous centrifuging, and washed cells were re-suspended in their respective larval culture tanks. This feeding was carried out every 24 hours. The larval culture was continued for 12 days, and during the experimental period the number of survivors, body length and body weight of the larvae were observed.

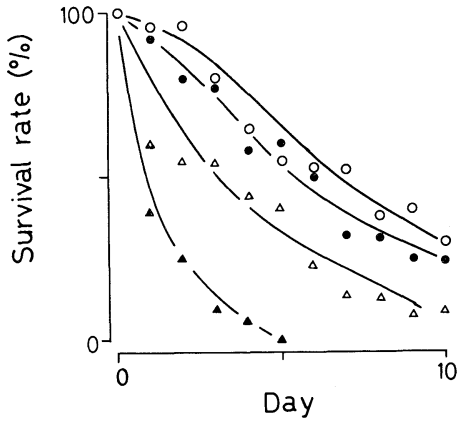

Fig. 2. Comparative effect of two strains of bacteria and *Chlamydomonas* sp. as food on the survival rate of *Artemia*. ○, *Chlamydomonas*; ●, B-9 strain; △, P strain; ▲, blank.

3. Results

Selection of bacterial strain as available food

As a result of the screening tests of the isolates as available food, 2 strains of *Acinetobacter* spp. (P and B-9) were found to be effective (Fig. 1). Especially effective was the B-9 strain, in which the survival rate of the cultured larvae was approximately 60 %. Survival of larvae fed on

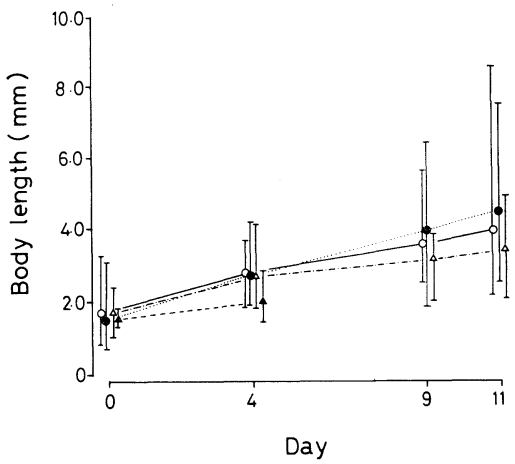

Fig. 3. Comparative effect of two strains of bacteria and *Chlamydomonas* sp. as food on the increase in body length of *Artemia*. ○, *Chlamydomonas*; ●, B-9 strain; △, P strain; ▲, blank.

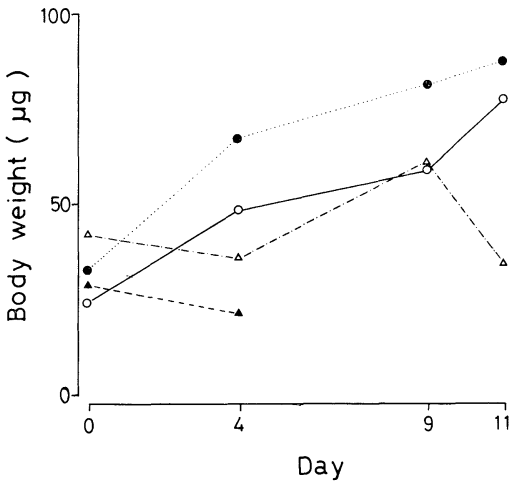

Fig. 4. Comparative effect of two strains of bacteria and *Chlamydomonas* sp. as food on the increase in body weight of *Artemia*. ○, *Chlamydomonas*; ●, B-9 strain; △, P strain; ▲, blank.

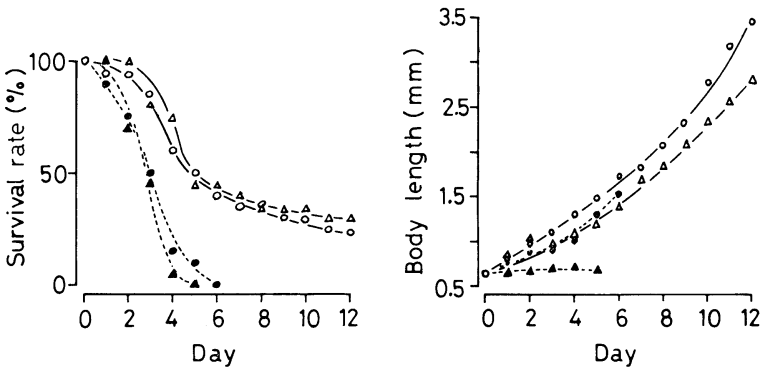

Fig. 5. Effect of different microbial foods, supplied independently, on the survival rate and body length increase of *Artemia*. Initial density of *Artemia* was one individual per m*l*. Cultivation temperature was 20°C. ○, B-9 strain (10^8/m*l*); ●, microbial flock (10^8/m*l*); △, *Chlorella* sp. (10^7/m*l*); ▲, non-addition of food.

Table 1. Effect of different food microorganisms, supplied independently or mixed, on the growth coefficient and survival rate of *Artemia salina*, cultured in a mass-culture tank.

Food density (cells/m*l*)			*Artemia* density (ind./m*l*)	Temp. (°C)	Growth** coefficient	Survival rate (%)
B-9 str.	Microbial flock	*Chlorella*				
10^8	—	—	1	20	0.05897	24
—	10^8	—	1	20	0.05800	0
—	—	10^7	1	20	0.05073	30
—	—	—	1	20	0.01503	0
10^8	—	10^6	1	20	(1) 0.06172*	50
					(2) 0.06375*	48
10^8	—	10^7	1	20	0.06132	45
—	10^8	10^6	1	20	0.04671	24
—	10^8	10^7	1	20	0.05840	40
10^8	—	10^6	5	20	(1) 0.06593*	54
					(2) 0.06941*	58
10^8	—	10^6	20	20	0.03068	20
10^8	—	10^6	50	20	0.02286	9
10^8	—	10^6	5	28	0.07195	38

* Results of the two different experiments.

** Regression coefficient of the curve obtained by plotting the ratio of the logarithm of body length to the time from the start of culture.

the P strain was about 30% up to 7 days, whereas using the other strains, the larvae could not survive during this experimental period.

Estimation of food value Larval culture using these two strains (P and B-9) with *Chlamydomonas* sp. as a control, was carried out. Survival rate of larvae fed on *Chlamydomonas* sp. was 31%, on B-9 was 26%, and on P was 9% (Fig. 2). The increase in body weight and body length of the larvae was greatest for the larvae fed on B-9, followed by *Chlamydomonas* fed larvae, and growth using the P strain was negligible (Figs. 3 and 4). The individuals forming eggs in the ovarium numbered, at 11 days, four with B-9, two with *Chlamydomonas* sp., and none with the P strain.

The above results show that the B-9 strain

is of slightly less value than *Chlamydomonas* sp. as available food for *Artemia* larvae.

Conditions for mass-culture To obtain large yields of *Artemia* larvae for practical mariculture purposes, it is necessary to use a large scale tank with an open system. The present experiments were carried out using a 500-*l* slate tank to examine the possibility of using bacteria as food for further mass-culture of *Artemia*.

Shown in Fig. 5 are the results of the first investigation, in which the survival rate of the larvae fed on *Chlorella* was highest, and increase in body length of larvae fed on the B-9 strain was greatest. Larvae fed on the microbial flock had almost all died by the 6th day, although the growth coefficient was equal to that of the larvae fed on B-9 (Table 1). The growth coefficient is the regression coefficient of the curve obtained by plotting the ratio of the logarithm of body length to the time from the start of culture.

In the second examination, as shown in Fig.

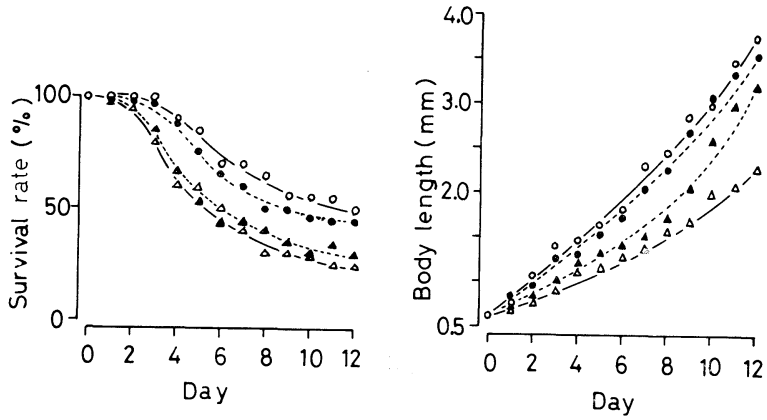

Fig. 6. Effect of combined microbial foods on the survival rate and body length increase of *Artemia*. Initial density of *Artemia* and cultivation temperature are the same as shown in Fig. 5. ○, mixture of B-9 strain (10^8/m*l*) and *Chlorella* sp. (10^6/m*l*); ●, mixture of B-9 strain (10^8/m*l*) and *Chlorella* sp. (10^7/m*l*); △, mixture of microbial flock (10^8/m*l*) and *Chlorella* sp. (10^6/m*l*); ▲, mixture of microbial flock (10^8/m*l*) and *Chlorella* sp. (10^7/m*l*).

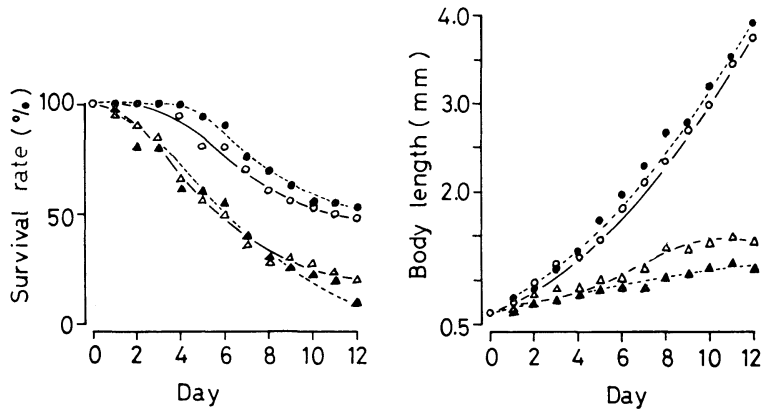

Fig. 7. Effect of *Artemia* density on their survival rate and body length increase. *Artemia* was cultured on a mixture of B-9 strain (10^8/m*l*) and *Chlorella* sp. (10^6/m*l*) at 20°C. ○, 1 indiv./m*l*; ●, 5 indiv./m*l*; △, 20 indiv./m*l*; ▲, 50 indiv./m*l*.

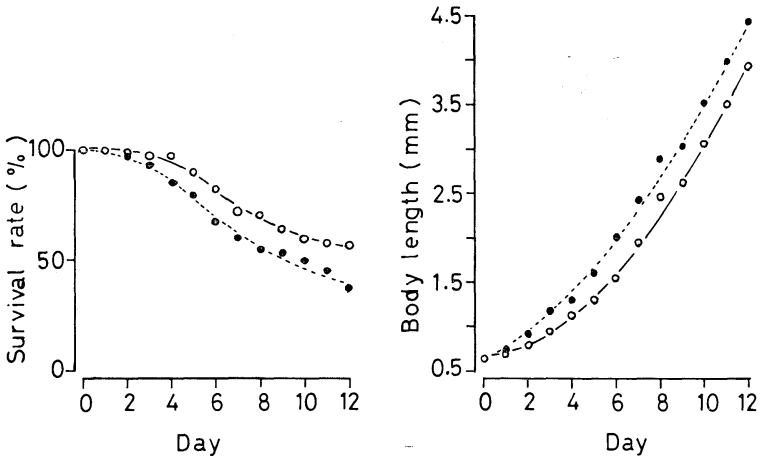

Fig. 8. Effect of cultivation temperature on the survival rate and body length increase of *Artemia*. *Artemia* (5 indiv./m*l*) was cultured on a mixture of B-9 strain (10^8/m*l*) and *Chlorella* sp. (10^6/m*l*). ○, cultured at 20°C; ●, cultured at 28°C.

6, the highest values for survival rate and body length increase were found in the larvae fed on the mixture of the B-9 strain (10^8/m*l*) and *Chlorella* sp. (10^6/m*l*).

From the results of the third examination, it is clear that the culture density of 5 indiv./m*l* produced the highest survival rate and growth coefficient (Fig. 7).

Furthermore, larval cultures at 20°C and 28°C indicated that survival rate was higher at 20°C and growth coefficient was the reverse (Fig. 8). This result suggests that mass-culture of *Artemia* as food for mariculture purposes is better carried out under low temperature conditions to increase survival, especially as *Artemia* eggs are becoming increasingly expensive.

4. Discussion

The results of the screening tests indicate that even in closely related species of bacteria there are differences in availability as food for *Artemia* larvae. This conclusion agrees closely with the data derived from studies of feeding experiments by PROVASOLI *et al.* (1959) in that *Tigriopus* and *Artemia* display a diverse ability to utilized different species of algae as food, and that this selectivity does not depend on the size of the food organism, as one organism may be nutritionally suitable, and the other unsuitable. The B-9 strain, which has a slightly lower food value than *Chlamydomonas* sp., was suitable for the larvae of the brine shrimp. Though our results provide experimental support for the belief that bacteria are available as food for marine animals (REISWIG, 1975; RIEPER, 1978), we consider microbial flocks which are made nutritionally more valuable by attaching an available protozoa or unicellular algae better as larval food for mariculture purposes than aggregates formed by the available bacterium alone.

Concerning the simple food experiments, it is not clear why the larvae fed on the microbial flock produced by the method of IMAMURA and SUGITA had almost all died by the 6th day even though their growth coefficient was equal to that of the larvae fed on the B-9 strain. Although there is no concrete nutritional evidence, we may speculate as to the reason as follows. One hypothesis which is supported by the results of the mixed food experiments is that the growth coefficient and survival rate of the larvae fed on the mixture of the microbial flock (10^8/m*l*) and the *Chlorella* (10^7/m*l*) are higher than those of the ones fed on *Chlorella* or the microbial flock only; that is, the larvae fed on the microbial flock received nutritional supplements from *Chlorella*.

On the other hand, the growth coefficient and survival rate of larvae fed on the mixture of the B-9 strain and *Chlorella* were higher with

a low concentration of *Chlorella* than with a high concentration, in contrast to the microbial flock and *Chlorella*. This result could have two explanations in that i) the relative food value of *Chlorella* is lower than that of *Chlamydomonas* sp. (SICK, 1976) or ii) high concentrations of *Chlorella* prevent the growth of *Daphnia* (RYTHER, 1954). Apart from the nutritional interpretation, the result that the food value was higher in mixed food than in simple food is explained by the finding of HANAOKA (1977) that ammonia accumulated in a closed system of cultivation is absorbed by *Chlorella*; that is, nutrient recycling in the tank was carried out by *Chlorella*.

Although these experiments were intended to provide criteria for the mass-culture of *Artemia* using bacteria as food, the data concerning the culture density, growth and survival rates of the larvae seem as yet insufficient. Hence, to use bacteria or microbial flock practically as food for mariculture purposes, further information about bacterial behavior in the culture tank will be necessary. We anticipate that bacteria will be found to be useful not only as food but also as biological controlers of fish disease and activators of the rate of nutrient regeneration.

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餌料細菌を用いるアルテミアの大量培養

安田公昭，多賀信夫

要旨: アルテミア（*Artemia salina* LEACH）の培養に従来用いられてきたクラミドモナス（*Chlamydomonas* sp.）やクロレラ（*Chlorella* sp.）などの餌料に代りうる有効な餌料細菌を選択するため，今村・椙田方式（1972）で生産される微生物フロック餌料から分離された細菌12株と酵母1株とを供試餌料として，アルテミアの培養が試みられた。その結果，*Acinetobacter* 属細菌の2株（B-9 およびP株）に餌料としての有効性が認められた。更に，B-9 菌株とクラミドモナスとを投餌した場合のそれぞれの餌料効果を比較した結果，アルテミアの歩留りは10日間で前者は 26％ であり後者は 31％であったが，その体長および体重の増加は前者の場合優れていることが判明した。

B-9 菌株とクロレラとの単独および混合餌料ならびに微生物フロック餌料を用いて，アルテミアの大量培養（500 *l*）に関する最適条件の検討が行なわれた。その結果，培養温度 20°C で，B-9 菌株とクロレラとの混合餌料をそれぞれ 10^8/m*l* および 10^6/m*l* の濃度割合として添加し，アルテミアの収容密度を5個体/m*l* として培養した場合に，アルテミアの歩留り（10日間で 60％）と体長増加は最良であることが認められた。