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Observations on the mass culture of photosynthetic sulfur bacteria (PSB) and their role as diet in the production of rotifer and brine shrimp

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Abstract

The paper deals with the mass culture of photosynthetic sulfur bacteria using organic fertilizer of groundnut oil cake. A concentration of 870-950 million cells/ml was achieved in three days of inoculam. The size of the bacterium was around 0.3-0.5 µ. PSB has a wide range of tolerance of salinity 25-40 ppt, temperature 25-35°C, and light. Brine shrimp was grown from nauplii to adult within 12 days and rotifer concentration of 202-210 nos/ml was obtained within 5 days with this bacterial diet. Significant difference in survival and growth of brine shrimp and rotifer concentration was not noticed between the microalgal and bacterial diets. The results of the study revealed that the PSB can be used as live food for the mass culture of brine shrimp and rotifer.

Utilization of bacteria as mariculture feed has been an area of international interest in recent years. Since most of the bacterial strain can be easily cultured on the waste products or using organic fertilizers, they attain a higher cell concentration at shorter period than those of microalgae species and yield a greater biomass (David *et al*, 1996). The continuous mass culture of microalgae is a constraint because it requires specialized facilities, fastidious growth, controlled temperature and light. So, the use of micro algae in the commercial hatcheries incurs considerable expenses. Even partial replacement of algal diets by bacteria could significantly reduce the production cost and enhance the maximum concentration of herbivorous zooplankton, larval growth and survival rate compared with those of fully algal fed cultures. For example, an addition of bacterial strain CA2 to axenic culture of oyster larvae fed *Isochrysis galbana,* enhanced the growth of oyster larvae, the proportion of larvae that set to produce spat and the subsequent size of spat (Phillippe *et al.* 1994).

Today most of the marine fish and shrimp larval stages in hatcheries are fed initially on rotifers for 10-20 days from egg and later on *Artemia* nauplii. A number of different feeds have been used to culture brine shrimp and rotifer. Generally rotifers are cultured by using expensive microalgae such as *Chlorella* spp, *Nannochloropsis* spp, marine yeast and the *Aartemia* by using baker's yeast and rice bran. The photosynthetic sulfur bacteria (PSB) belongs to the family Athiorhodaceae of the genus *Thiobacillus*. They are spherical in shape and the coccus form contains bacteriopurpurin and

are light sensitive under anaerobic conditions. They are mass cultured and used as the diet for the mass production of herbivorous zooplankton.

The present investigation was carried out to observe the mass production of PSB and their utility as feed in the mass culture of rotifers and brine shrimp. The results obtained are presented in the paper.

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Material and methods

Techniques for mass production of PSB

The filtered seawater at a salinity of 32 ppt, pH 8.45 and temperature of 30° C was pumped into one tonne tank and fertilized with 400 g of groundnut oil cake. Vigorous aeration was provided from the start of the culture. After 72 hours of fertilization, the colour of the water in the culture tank changed to pink indicating the development of PSB. The PSB was inoculated at the rate of 0.02 million cells/ml into another one tonne tank having the same environment for the culture experiments.

Culture of brine shrimp using PSB as diet

Freshly hatched *Artemia* nauplii (San Franciscan strain, USA) were reared in three round bottom cylindrical glass containers of 10 1 capacity each with volume of 8 litres of filtered seawater at 30 ppt salinity, 8.40 pH, 3.8 ml/l dissolved oxygen and 30°C temperature. Each container was introduced with *Artemia* nauplii at the rate of 300 no's/litre. In order to deplete most of the yolk food reserves of the nauplii, they were fed with PSB after 72 hours of hatching. The feed was given once in a day at a concentration varied between 5.07 and 5.32 lakhs/ml. The food concentration was increased in proportion to the growth of *Artemia*. Half of the culture medium was changed every third day with 100 ppt high saline water and survival, developmental stage and total body length (20 animals) of the *Artemia* were determined. The experiments were conducted for 13 days.

Mass culture of rotifer (*Brachionus plicatilis*) using PSB as diet

About 500 litres of PSB in exponential growth phase with the cell density of 742 million/ml were transferred into 3 tanks of one tonne capacity each with water salinity of 32 ppt, and 8.38 pH, 0.75 ml/l oxygen and 31.5°C temperature. About

150 lakh rotifers were iccntroduced at the rate of 30 no's/ml. Vigorous aeration was provided. Increase in count of rotifer and the environmental parameters were checked at 48 and 72 hours and 200 litres of PSB was added to maintain the optimum feed concentration. After 120 hours (5 days) final count was taken for result. The O_2 content of culture medium when the rotifer reached the maximum concentration was 3-5 ml/l.

The rotifer population counts were made from five 1 ml aliquot samples collected randomly, fixed with a drop of lugols solution and counted under microscope using zooplankton counting chamber.

Results

Production of photosynthetic sulfur bacteria (PSB)

The PSB were found to grow well at the salinity range of 30-34 ppt. The temperature ranged between 28-34°C. The initial pH of the culture medium was 8.4(before fertilization) and reduced to 6.7 within 24 hours of fertilization. The pH again raised steadily up to 8.38 in proportional to the growth of bacteria. The oxygen was completely absent during the growth phase of the culture period. Fig. 1 shows the growth of bacteria after inoculation in one tonne culture tank. In the maximum growth phase of PSB, the culture medium became dark pink in colour and remained stationary for 3-5 days. The maximum concentration achieved was 870-950 million cells/ml with in 3 days of inoculation.

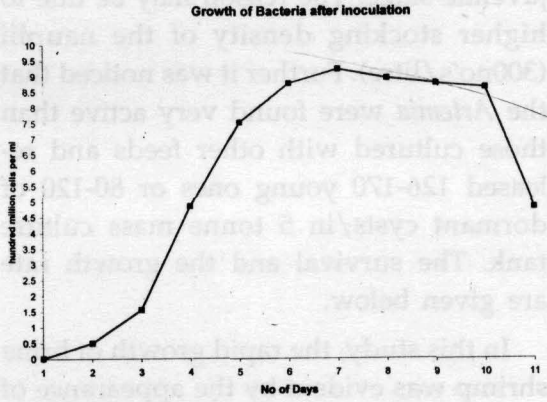

Fig 1. *Growth of Bacteria after inoculation*

Table 1. *Growth of Artemia using PSB as diet*

Days	Temperature C		Salinity ppt	pH	Survival No/ml	Size Observation Range
0	26.8	33	8.32	0.3	450-550 Micron	Newly hatched nauplii 300/lt
2	27.5	33	8.25	0.25	1-1.5 mm	Pre juvenile stage
5	29.8	55	8.28	0.24	4-5 mm	Juvenile stage 50% of the water changed using high saline water.
7	26.7	55	8.16	0.22	6-7 mm	Pre adult stage 50 % of the water changed using high saline water.
10	29.9	70	8.21	0.21	7-9 mm	Pre adult and adult stage
11	29.7	70	8.27	0.21	8-10 mm	Do

Culture of brine shrimp using PSB as diet

In the replicate trails conducted using PSB as the sole diet, the survival rate varied between 65 and 70.4% from nauplii to adult. For enumeration in experiments pre-adults were defined as about 7mm and above 5 mm, adults are 8mm and greater in size. The total length have been measured as the distance from head to the tip of the tail. It was observed that 70-75% of the total population became adult and the remaining were in pre-adult and juvenile stage. The reason may be due to higher stocking density of the nauplii (300no's/litre). Further it was noticed that the *Artemia* were found very active than those cultured with other feeds and released 126-170 young ones or 80-120 of dormant cysts/in 5 tonne mass culture tank. The survival and the growth rate are given below.

In this study, the rapid growth of brine shrimp was evident by the appearance of 7-9 mm animals and copulating pairs (Riders) within 9-10 days. Microscopic observation of the young ones (day 3 onwards) and adults of the intestinal tract, gut and their faecal matters showed mostly pink, intact bacteria cells.

Culture of rotifer using PSB as diet

The water quality parameters in the culture experiments ranged from 32-33 ppt for salinity, 29-33°C for temperature and 8.01-8.38 for pH. The maximum concentration of rotifer varied from 201-210 no's/ml within 5 days after the introduction of rotifer.

The Fig. 2. shows the multiplication of rotifer in different culture period. Rotifer density was determined by using zooplankton counting chamber. Triplicate sampling was done and average value was taken as result.

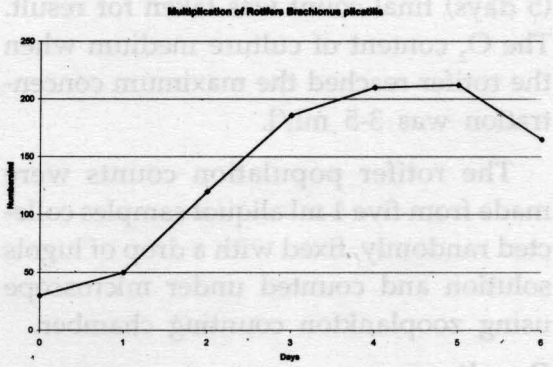

Fig. 2. *Multiplication of Rotters Brachionus plicatils*

Discussion

There is evidence that bacteria may play an important role not only in the regeneration and consumption of nutrients in the water column but also as food for direct utilization by herbivorous zooplankton. The formation of lipid by bacteria is dependent upon the nature of carbon compounds present in the culture media (Sallee, 1948). In the PSB culture tank, accumulation of hydrogen sulphide in the culture medium within 24 hours of fertilization may initiate the reproduction of the photosynthetic sulfur bacteria present in the seawater.

In the present study, the initial oxygen content of the culture medium was

5.5mg/l and observed to be nil after 24 hours of fertilization and maintained up to the maximum growth phase. The same was observed at 1 mg/l in the start of the declining phase. This clearly shows that PSB multiply in the absence of oxygen. Sallee (1948) stated that the formation of ammonia is effected with the deaminization of aminoacid present in the culture medium. In the present study the initial ammonia content in the culture medium was found nil and then ranged from 0-0.5 mg/l during the growth phase of PSB. In declining phase, the ammonia content raised up to 10 mg/l due to deaminization of the aminoacid present in the culture medium which is in agreement with Sallee (1948).

The PSB is very important microbial resource that can utilize small molecular organic substance. It is mainly used in fish, shrimp, scallop, pet fish breeding and the animal husbandry. PSB is rich in protein, vitamins, co-enzymes and vital material. Crude protein content of photosynthetic bacterium, *Rhodopseudomonas palustris* was about 72-74%. For the better growth and reproduction, brine shrimp and rotifer require linoleic (18:2n-6) and linolenic (18:3n-3) acids, which occur naturally in most of the bacteria, rice bran and soya bean oil.

Mass culture of rotifer depends upon the large-scale production of micro algae as food in commercial hatcheries. The present investigation proved the possibility of mass production of rotifer using PSB as the sole diet. James and Tawfiq (1998) reported the maximum concentration of 194-240 rotifers/ml in 5 days using *Chlorella*. Kandasamy *et al.* (1996) stated 225-260 rotifers/ml in 5 days using *Nannochloropsis salina* as food. Rotifer concentration of 400 individual/ml was obtained by Walford and Lam (1992) in bakers yeast feed within 10-14 days of culture period. The mass culture of rotifer using micro algae is extremely costly. In addition, it is an unreliable source of food as algal cultures are sometimes unstable and the *Chlorella spp* sometimes secrets the poisonous substances in the culture medium causes the sudden collaps in rotifer culture systems. The disadvantages of using bakers yeast as a substitute for micro algae are that the rotifer culture could not be maintained for longer period with bakers yeast alone and had lower reproductive rates than those cultured in micro algae. Regular harvesting when the rotifer density reached about 200/ml may have been an important factor in the control of rotifer population and maintenance of good culture conditions in the tanks. Vitamin B_{12} is found to be essential for rotifer growth and bakers yeast was deficient in this vitamin. The phytoplankton or bacteria may provide a source of the nutrients such as vitamin B_{12}, not supplied by bakers yeast. Based on these observations, a simple and inexpensive method for culturing rotifer, the PSB was introduced as a substitute for micro algae.

Although this study revealed the encouraging result in the culture of brine shrimp and rotifer using PSB as the sole

diet, further investigation is essential on the biochemical composition especially the PUFA content present in the PSB to use it in shell fish, molluscan and finfish hatcheries as larval food.

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