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Formulation of Aquafeed Using Purple Non-Sulfur Bacteria Isolated from Sundarban Delta and Freshwater Pond

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ABSTRACT

Aquafeeds contain high amount of protein. Formulation of aquafeeds with novel protein sources like dried microbial biomass alternatively called Single cell protein can contribute to a more sustainable Aquafeed Industry. Microbial protein sources of Purple non sulfur bacteria are a well-known fact and that may be exploited as a supplement in aquafeed. For this purpose, enrichment of Purple Non-Sulphur Bacteria (PNSB) was done using water samples collected from Sundarban Delta, West Bengal and another sample collected from Local fresh water pond in Kolkata. They were enriched in specific medium in microaerophilic lighted condition (10000 lux). Enriched samples were screened on the basis of greater protein and carbohydrate content and sample SG2 and FP was selected for formulation of fish feed. Selected samples SG-2 and FP shows Protein content of 10.115 and 10.579 µg/ml and Carbohydrate content of 236.4 and 220.8 µg/ml. Fish feed was formulated with biomass of selected PNSB samples mixing with specific binder materials and was fed to batch of fishes (*Xiphophorus helleri*) in aquarium culture. 4 aquarium was maintained feeding with i) Commercial fish feed, ii) Experimental fish feed containing both SG2 and FP, iii) Experimental fish feed with SG 2 only and iv) Experimental fish feed with FP only. Better survivality and growth of the experimental fishes was reported in the set ii) that is fed with Experimental fish feed containing both SG-2 and FP. Fish feed produced with PNSB having potential as a substitute of commercially available fish feed in the market.

Key words: Purple non sulfur bacteria, Fish feed, Aqua culture, Protein, Carbohydrate, Microaerophilic

Microbial variety is certainly considered one among the ardous regions of biodiversity studies. A considerable exploration is needed for knowing-how the biogeography community, assembly, and ecological processes, ameliorate for separating and figuring out new and factual microorganisms, having excessive specificity for diversified compounds [1]. Purple Non-Sulphur Bacteria (PNSB) is widely distributed in the ocean, lakes, rivers, soil and activated sludge also in high temperature, low temperature, low salt, and high salt environment. They grow in both micro aerobic and anaerobic light conditions while utilizing various substrates as sources of carbon and energy with ammonium and or/nitrate as a source of nitrogen and may also use sulphide or thiosulfate as an electron donor under photosynthetic condition [2-3]. They are metabolically the most versatile among all prokaryotes [4]. Phototrophic bacteria for waste treatment have generally proven to be cost effective system. This because PNSB does not only produce quality effluent, but produces substances of commercial interest such as single cell protein (SCP), biopolymers, antimicrobial agents and therapeutic compounds etc. Due to these, PNSB have been utilized by other researchers to treat different types of wastewaters such as concentrated latex wastewater [5], odorous swine wastewater [6], oil containing sewage wastewater [7-8], studied the influence of photosynthetic on calcite precipitation.

Phototropic bacteria are very dominant in polluted areas because they are very efficient in converting wastes into useful products [9-10]. Phototrophic bacterial cells are rich in proteins, vitamins and amino acids. The biomass of phototrophic bacteria reported to be highly nutritive by workers such as Kobayashi and Kobayashi [11], Shipman *et al*. [12], Sasaki *et al.* [13]. When grown in cheap organic wastes, they offer a good option to substitute the expensive fish and prawns' feeds [14]. Also, phototrophic bacterial cells are said to contain physiologically active substances/chemicals that acts as probionts.

The microbial biomass used as a dietary protein source for feed, also called microbial protein or single-cell protein, offers a sustainable alternative to the commercially available feed [15]. There is an increasing interest in the use of probiotics in aquaculture industries in response to the challenging environment. In general, the use of these beneficial bacteria in mainly designated for improvement of aquatic environment quality [16-18]. However, in recent years, a few studies reported the use of diverse variety of probiotics as supplement to improve nutritive values of aquaculture feeds [19]. Purple non-sulfur bacteria (PNSB) are promising microorganisms

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which can be used as feed as a source of single cell proteins or microbial protein [20-26]. Nevertheless, the application of probiotics in aquaculture is normally hindered by the increased production cost due the expensive price of commercial products. Fortunately, there exists the potential bacterial candidate such as Phototrophic bacteria, which is also known as Purple Non-Sulphur Bacteria and widely distributed in nature.

MATERIALS AND METHODS

Sample collection: Samples were collected from Sundarban Delta Region (from Godkhali and from Pakhiralay) and from local pond. The Malic Acid Yeast Extract Media was used for culturing Phototrophs (PNSB). Samples collected from Sunderban Delta and local pond were marked on the Screw-capped Bottles as: SG-1 (+NaCl), SG-2, SP-1 (+NaCl), SP-2, FP.

Enrichment of Purple Non-Sulphur Bacteria (PNSB): The samples collected from different locations were added in Malic Acid Yeast extract Medium into screw-capped tubes under lighted (10,000lux), microaerophilic condition. The bacteria were enriched for 3-4 days.

Protein extraction and estimation assay: The protein content of the microbial cells were estimated by Folin-Lowry Method [27] and the data was compared with the Standard Curve of Protein (BSA) for quantification.

Carbohydrate Extraction and estimation assay: The carbohydrate content of the microbial cells was estimated by Duboi's method [28] and the data was compared to a standard curve of glucose for quantification.

Harvesting cell biomass from bulk culture: After growing the selected sample in the media for about 7-10 days, the prominent growth was seen as the inoculated media turns dark red. The cell mass was harvested by centrifugation at 6000g for 10 minutes and was washed with 0.9% saline solution for 2 to 3 times.

Preparation of fish feed: Whole meal wheat flour was taken in a petri dish according to the cell-mass of the sample. Pellet Sample was diluted by adding very small amount of 0.9% NaCl. According to the cell-mass of the samples, flour was mixed with the cell-mass in 1:10 ratio. Dough was prepared with this sample solution, then the dough was flattened with the help of a centrifuge tube or rolling pin. Beads were cut out using cork-borer, then the beads were dried by keeping them overnight in hot-air oven at 60°C. Next day beads were stored in airtight container and kept in -20°C for future use.

The growth of the fishes was observed over 2 months: The length, breadth and weight of the fishes were measured after administration of the prepared fish-feed with PNSB as well as commercial feed.

RESULTS AND DISCUSSION

Water and sludge samples collected from the Delta region and local pond were enriched and after enrichment they showed different shades of red coloration from brown to blood red. Their shape, size, Gram nature, motility showed that their shape varies from rods to spiral, all were Gram negative and were motile.

The overall Protein estimation of the enriched five samples were done, of which two samples SG-2 and FP were high protein producers. Among them FP produces protein higher than that of SG-2, whereas, the sample SG-2 produces a very high cell mass. These high protein producers were also designated as Single Cell Protein (SCP). The yield of Protein in SG-2 and FP were very high. Phototrophs have been reported to contain high protein with essential amino acids and less amounts of nucleic acids [29]. They reported that PNSB are highly resistant to toxicants and contain 70-72% of crude protein and the composition of essential amino acids of these bacteria are similar to that of soya bean proteins. The overall Carbohydrate estimation of the enriched five samples were done, of which two samples SG-2 and FP were shown to be high carbohydrate producers. Among them SG-2 produces very high amount of carbohydrate 236.4 µg/ml (Table 1). On the basis of protein and the carbohydrate content, two samples SG-2 and FP were selected and were used in the preparation of the Experimental aquafeed.

Table 1 Estimation of carbohydrate, protein and dry cell weight of the enriched PNSB culture

Enriched culture	Cell dry weight (gm./l)	Protein (µg/ml)	Carbohydrate (µg/ml)
SG-1 (+NaCl)	73.57	8.725	133.2
SG-2	132.14	10.115	236.4
SP-1 (+NaCl)	86.57	8.262	106.5
SP-2	76.42	8.416	50.7
FP	102.85	10.579	220.8

SG: PNSB enriched from Sundarban Gadkhali area (brackish water), SP: PNSB enriched from Sundarban Pakhiralaya (Brakish Water), FP: PNSB enriched from fresh water pond Kolkata surrounding area

The growth and rate of survival of the batch of fishes (*Xiphophorus helleri*) upon the administration of PNSB-incorporated fish feed was the foremost observation of the experiment. The fishes were fed two times a day. The results were seen that the growth rate of the fishes in the Experimental feed tanks were better than that of the commercial one (Table 2). The mean length, breadth and weight of the fishes were observed over the period of two months of the experiment.

Table 2 Growth of *Xiphophorus helleri* (Red molly/Swordtail fishes) over 2 months

Aquariums	Day 0 (average)	1 week (average)	2 months (average)
Aquarium-1 (Commercial feed)	Length (cms) 4.2	Length (cms) 3.9	Length (cms) 4.8
	Breadth (cms) 1.0	Breadth (cms) 1.0	Breadth (cms) 1.2

	Weight(gms) 0.81	Weight(gms) 0.84	Weight (gms) 1.60
Aquarium-2 (experimental mix SG-2+FP)			
	Length(cms) 4.0	Length(cms) 4.1	Length (cms) 4.6
	Breadth(cms) 1.1	Breadth(cms) 1.3	Breadth (cms) 1.3
	Weight (gms) 0.86	Weight (gms) 1.07	Weight (gms) 1.67
Aquarium-3 (experimental SG-2)			
	Length (cms) 3.8	Length (cms) 4.0	Length (cms) 4.5
	Breadth (cms) 0.9	Breadth (cms) 1.1	Breadth (cms) 1.7
	Weight (gms) 0.84	Weight (gms) 0.99	Weight (gms) 1.49
Aquarium-4 (experimental FP)			
	Length (cms) 4.14	Length (cms) 4.2	Length (cms) 0.0
	Breadth (cms) 1.12	Breadth (cms) 1.08	Breadth (cms) 0.0
	Weight (gms) 0.93	Weight (gms) 0.99	Weight (gms) 0.0

After observing the rate of survival of fishes (*Xiphophorus helleri*) over one month it was seen that the survival rate of the Red Molly fish was 81.25% in the aquarium that was fed with the mixture of the prepared Experimental fish feed (SG-2+FP) (Fig 1). Even after 2 months the rate of survival of the mixed Experimental feed (SG-2+FP) was high 56.25%, as compared to other feeds. But the fishes of the aquarium fed with Experimental FP was observed very low as compared to other aquariums and after two months there no fish survived in this aquarium. The weight gain by the fishes were also high in the period of experiment.

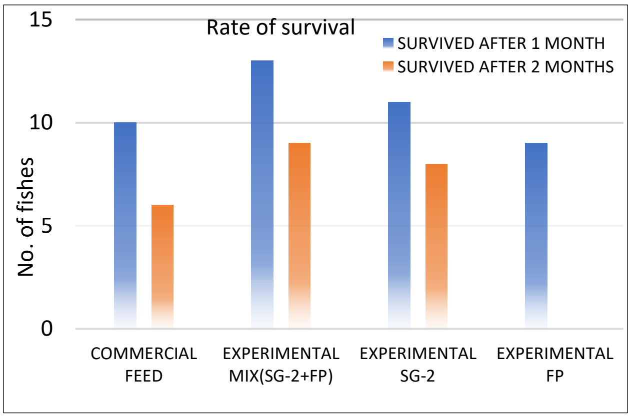

Fig 1 Rate of survival of fishes observed over selected period of time

The rate of Survival of fishes fed with the Experimental mix (SG-2+FP) was 81.25% lesser than the PNSB *Rhodovulum sp*. having 86.7% [30]. The tiger sharks were fed with PNSB isolated from the east Kolkata Wet land that produced the 0.16% of protein (%CWD) [31]. Studies on enhanced performance of freshwater or marine fish when their diets are supplemented with bacteria, as is the case in Nile tilapia *Oreochromisni loticus* [32] and freshwater prawn [33] had been seen. Alloul *et al*. [20] reported the importance of supplementation of purple bacteria namely different species of *Rhodopseudomonas, Rhodobacter* in feed of white leg shrimp (*Penaeus vannamei*) and investigated the shrimp's tolerance against *Vibrio* and ammonia stress. Again, *Rhodobacter sphaeroides* strain UMS2, grown utilizing fish hatchery waste, was found to be used as feed supplement in *Tubifex* Sp [34]. On the other hand, Nicolas *et al*. [35] observed that prey items with a high bacterial load were rejected by turbot larvae, potentially leading to starvation.

CONCLUSION

In recent years phototrophic purple bacteria are seen to be an increased interest in aquaculture industry as it able to satisfy the increasing demand for affordable, safe and high quality of feed ingredient. The beneficial effects of these bacteria are known in different areas especially as feed supplement because they are rich in protein, lipid, minerals, carotenoids and other biological cofactors. The addition of bacterial cell in the fish feed of various cultured fish species have been investigated by a number of researchers. The findings of the present study reveal that enrichment culture of purple non sulfur bacteria having efficacy as the fish feed supplement or having potential as aquafeed as they are good source of protein and carbohydrate. Again, this group of organisms having potential to grow in waste materials which may be a cost-effective approach to reduce the pollution load as well as production of Industrially important materials.

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