Use of Probiotics in larval rearing of new candidate species

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India is a rich country blessed with enormous wealth of natural resources but unfortunately both terrestrial and aquatic resources are declining due to various anthropogenic stresses. On one hand our aquatic resources are dwindling and we are losing precious genetic resources and on the other hand neither do we have a contingency plan nor we have an appropriate technology for culture of most of the endemic cultivable food and ornamental species. One solution to this problem may be the domestication and culture of new candidate species, which include *Labeo gonius*, *L. dero*, *L. calbasu*, *Notopterus* sp., *Tor* sp., *Mystus* sp., *Pangasius pangasius*, *Lates calcarifer*, *Mugil cephalus* and other endemic ornamental fishes and shrimps.

There are several bottlenecks in achieving the target of profitable culture of new candidate species. The larval rearing of these fishes in captivity is not so easy. In most experiments survival rate is very poor. The reasons may be food, water nutrients and/or diseases. In India the average survival rate of shrimp larvae is reported to be around 25-30 % while in Thailand the average survival is around 50-60 %[1]. Hence for success in culture of candidate species we must ensure quality feed, good environment and disease free seeds and juveniles. Antibiotics have sometimes been used to reduce disease, however indiscriminate use has in some cases led to increased antibiotic resistance and problem of tissue residues and trade issues[2,3]. Vaccines are successfully used in other livestock industries but there are no vaccines currently available for most of the fish diseases in this region. So how can we prevent disease and improve the growth of fish at aquafarms ? Obviously effective farm management practices are crucial and there are many management issues that need to be addressed. However, one measure that might be of assistance is the use of "probiotics".

Probiotics are a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal balance and health of the host[4]. The first probiotic discovered long time ago was *Lactobacillus* sp., the lactic acid producing bacteria. They were thought to prevent colonization of the gut by other disease causing bacteria - a process known as competitive exclusion. Presently the range of probiotics extends well beyond the *Lactobacillus* sp. to include *Bacillus* sp., *Vibrio* sp., *Pseudomonas*, yeasts and algae. Some of the commercial probiotics currently available include Aqualact, Probe-la, Lacto-sacc Epicin, Biogreen, Environ, Wunopuo- 15 and Epizyme. Feed probiotics are applied with the feed and a binder (egg or cod liver oil) and most commercial preparation contain either *Lactobacillus* sp. or *Saccharornyces cerevisiae*.

The characteristic feature of a probiotic microbe is it should be able to colonize the gastro-intestinal tract, but the intestinal microflora in aquatic animals changes rapidly with the constant influx of microbes coming from water and food. The microbial community of the gut can therefore be considered to be transient in nature. This transience allows the extension of the probiotic concept to the use of live microbial preparations in ponds. Therefore in aquaculture it is difficult to delink probiotics from bioremediators[5].

Probiotics also show antagonism to other organisms through: i) competition with other species for binding (colonization) sites; ii) specific antagonism against other species; and iii) nonspecific antagonism against other species. Probiotic bacteria may stabilize the intestinal microflora and the principle of bacterial antagonism applies to the preparation of new probiotics as well. Some of the antagonistic bacteria against fish and shellfish pathogens include *Bacillus* spores, *Roseobacter* sp., *Cornobacterium divergens*, *Pseudomonas* sp., *Alteromonas*, and *Pseudoalteromonas* against pathogens like *Vibrio harveyi*, *V. anguillarum*, *Edwardasiella*, *Aeromonas*, and *Pastrella*[6].

The composition of the intestinal flora of the larvae from first feeding onwards plays an important part in the defense against colonization and growth of opportunistic pathogens. Maeda and Nagami (1989) reported decline in pathogenic Vibrio in larval tanks of prawns and crabs by using probiotics. Other studies on the role of probiotics in shell fish culture include the work done by Douillet and Langdon[7], Maeda and Liao[8], Uma et al.[9], Vasudevan[2], Sridhar and Paul Raj[10], and Anikumari et al.[11]. The role of probiotics in health management of fish has been studied by Baird[18], Robertsen et al.[12], Metaillier and Hollocou[13], Gopalannan et al.[14], Mohammad[15], and Karunasagar[5].

Most of the probiotics have been isolated from seawater such as the unicellular marine alga *Tetraselmis suecica* and another marine alga *Ulva fasciata*. Several bacteria have been examined as potential probiotics for fish. While using the probiotics for effective disease control one must take care in their choice because some of them may be potentially pathogenic for aquatic animals eg. *V. alginolyticus* and as we still don't know the precise mode of action of these organisms, it is essential to ensure that the organism (probiotic) is harmless to the host.

A survey of Nellore district (Andhra Pradesh) which has 31 giant freshwater prawn culture ponds and several shrimp culture facilities[16] has revealed that farmers are using both water and feed probiotics. The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. licheniformis*, *Nitrobacter* sp., *Aerobacter* sp. and *Saccharomyces cerevisiae* etc. and feed probiotics contain *Lactobacillus* sp., *Bacillus* sp. or *Saccharomyces cerevisiae*. The farmers claim that these probiotics improve the growth of shrimp larvae in initial period up to 50 days of culture. Survival of larvae is also reported to be better[11].

Regular use of probiotics in feed of fish, in U. K. and other European countries has been reported to have several health benefits. In shrimp hatcheries it is reported to have controlled the high incidence of diseases in larvae and led to dramatic improvement in shrimp health. Atlantic Salmon fed with probiotics enjoyed increased survival and reduced mortality caused by Vibriosis, Furunculosis and Enteric Redmouth diseases. Moreover, fish showed enhanced appetite, grew better and had lessr problems with fin and tail rot. *Vibrio alginolyncus* introduced in larval rearing tanks caused a reduction in the incidence and severity of luminous vibriosis caused by *Vibrio harveyi* and improvement in growth of shrimp larvae[17].

Indian fish pathologists are also looking at probiotics as a potentially useful disease prevention measure in aquafarms and active research is continuing. The Cochin University of Science and Technology, Kochi, Kerala has taken lead in the research on probiotics. Other National Institutes like Central Marine Fisheries Research Institute, Kochi, College of Fisheries, Mangalore, Kamataka, National Institute of Oceanography, Goa, Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujrat and some universities are also involved in research on potential probiotics.

In nutshell, we can say that there are many positive reports about the use of probiotics, particularly in feeding of larvae from first feeding onwards. Probiotics are a welcome addition to the armament of disease prophylaxis in aqua-farms although the technology and science behind it is still very much in a developmental phase. It seems likely that the use of probiotics will gradually increase and that success of aquaculture in future may be synonymous with the success of probiotics, which, if validated through rigorous scientific investigation and used wisely, may prove to be a boon for the aquaculture industry.

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Farmers stack the soil while excavating pond in the farm site and then, when it is high enough, they construct the overhead tank on the stack. This is an effective way to reduce the cost considerably as it does not involve construction of any tower or pillar. (Fig. 2).

Rejection of the breeding pool concept

A survey on 2000 to assess the current status of hatcheries in Bengal, revealed that the farmers have totally rejected the breeding pool approach and instead both breeding and hatching is done in Chinese-style hatcheries. The farmers are of the opinion that the sloping towards central outlet prevents the total mixing of milt and egg and there by reduces fertilization rate. Better fertilization rates are achieved when spawning is done in Chinese hatcheries as the floor surface is plain, which permits easy mixing of milt and eggs.

In some farms that I have observed the hatcheries are located within the grow out pond. This not only requires less supply of water to hatcheries but also reduces the cost of supplying a constant flow to hatcheries. (Fig. 3).