Probiotic-based cultivation of *Clarias batrachus*: importance and future perspective

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ABSTRACT *Clarias batrachus* (Linn.) is widely recognized in Indian sub-continent for its nutritional and economic significance. At present, it remains at a merely vulnerable state. Pathogenic infections, diminution of natural habitats and introduction of allied exotic fishes are the causes of productivity constraint, particularly in Southern Asia. Conversely, African cat fish *Clarias gariepinus* has been significantly identified as a potential threat to biodiversity, despite being its large scale cultivation across the world. Thus emphasis on indigenous *C. batrachus* farming is becoming inevitable. Currently, screening of autochthonous probiotic organisms for the cultivation of *C. batrachus* in semi-intensive manner is getting importance. At the same time, molecular omics-based technologies are also gaining considerable attention to identify potential probiotic markers. This review provides an overall concept of probiotics, its application and future perspectives in relation to the cultivation of *C. batrachus*.

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Introduction

Aquaculture is becoming a growing and vibrant sector to provide food security to a large population of rural mass. Southern Asia, one of the mega biodiversity hotspots, is native to many indigenous freshwater fish species. *Clarias batrachus* (Linn.) (Asian catfish) is one of the most sought-after aquatic products owing to its nutritional benefits and economic significance though the production of it remains low as per the major carps are concerned. The species is presently on the verge of extinction in Southern Asia due to exploitation of its natural habitats, reclamation of wetlands, uncontrolled introduction of allied exotic fishes and infectious diseases caused by bacterial pathogens (Ahmed et al. 2012). Abrupt use of pesticides in the adjacent agriculture field also has made the situation more hostile. In aquaculture, prevention and control of aquatic diseases by chemical additives or antibiotics may generate antibiotic-resistant bacteria (ARB) and thus creates a serious concern to public health (FAO 2006). The bacterial isolates obtained from a *C. batrachus* population exhibited an increasing order of resistance against antibiotic colisitin, ampicillin, gentamycin, carbenicillin, tetracycline, streptomycin, and ciprofloxacin (Pathak and Gopal 2005) that may pose risk to fish fauna and public health (Hoseinifar et al. 2017). Transmission of antibiotic-resistant genes may thus lead to the expansion of pathogenic populations (Bäumler and Sperandio 2016). There are many reports regarding a sharp decrease in productivity due to abrupt use of anti-microbial drugs (Alcaide et al. 2005). Probiotics are beneficial microorganisms which (as ecofriendly and biocompatible substances), are also in increased use to prevent and control aquatic diseases in recent decades. They confer protection against pathogens; e.g., by production of bacteriocins, siderophores, lysozymes and other antimicrobial compound (Ige 2013). Furthermore, they may stimulate immune responses of the host (Bandyopadhyay and Das Mohapatra 2009). Probiotics can be used as a functional feed additive to enhance feed digestibility and fecundity (Hoseinifar et al. 2017).

Scientific approaches along with social and ecological awareness need to be adapted to rescue *C. batrachus* from the existing deplorable state. Semi-intensive mode of cultivation must be prioritized for the conservation of native *C. batrachus* (ICAR-CIFA, 2016-17). Currently, use of autochthonous probiotics is gaining increased importance worldwide to cultivate the species in semi-intensive manner.

Aquaculture probiotics

Probiotics are live microorganisms that confer health benefit to the host when administered in adequate amount (FAO/WHO 2001). A probiotic should be non-pathogenic...
and non-toxic to the host and must not contain any virulent or antibiotic-resistant gene (Fuller 1989). It should remain viable without genetic alteration for prolonged periods under storage and field conditions. However, competition for nutrients and production of inhibitory substances could occur even in the rearing water (Dalmin et al. 2001). The acid and bile tolerance property may enable the probiotic organisms to colonize the intestinal tract of the host (De et al. 2014). A probiotic must also exhibit high cell-surface hydrophobicity which ensures its capacity of adherence to the intestinal wall (Krasowska and Sigler 2014). It should be target specific and must reach the desired location (Wang et al. 2008).

Probiotics have been found to play a significant role in the sustainable development of aquaculture through different approaches (Table 1). Aquaculture probiotics do possess different attributes from terrestrial-based probiotics as the gastrointestinal microbiota of aquatic species is affected by the flow of water passing through the digestive tract (Gatesoupe 1999). Commercial probiotics are available both in dry and liquid forms. The dry probiotics (Table 2) consist of spore-forming microorganisms, binding material, a cascade of enzymes coupled with vitamins and other functional additives. The ingredients are then mixed with sterile water for brewing at 27-32 °C for 16 to 18 h with continuous aeration (Sahu et al. 2008). Alternatively, it can directly be added to the feed to use in the same day. However, the hatcheries mostly prefer liquid forms (Table 3) than the dry, spore-forms. Generally, commercial liquid probiotics are extremely hygroscopic and need to be kept away from moisture and sunlight. These liquid forms are applied directly to culture water in the morning and evening and have faster mode of action (Sahu et al. 2008). The immersion method of storing live fish in a probiotic-rich container for certain period of time on a regular basis is also gaining increased attention (Feliatra et al. 2018).

**Molecular tracking of probiotic markers**

Molecular identification technologies (e.g., proteomics, transcriptomics, secretomics, metabolomics, interactomics) are getting priority over traditional approaches in recent times to decipher the fundamental basis of probiotics functionality (Papadimitriou et al. 2015). Systematic study of functional genomics is crucial to properly validate putative probionts (Fig. 1). Several attempts have been made to identify molecular markers that would facilitate the rapid screening of probiotic strains. The probiotic must survive at high intestinal bile salt concentration through increased expression of bile stress-regulatory genes (e.g., bsh), molecular chaperones (e.g., GroES, DnaK), proteases (e.g., Clps) or DNA repair proteins (e.g., uvrB) (Papadimitriou et al. 2015; Hamon et al. 2014). Tripathy et al. (2014) observed several probiotic marker genes including fibronectin binding protein (fbp), mucus binding protein (mbp) and bile salt hydrolase (bsh) in *Lactobacillus plantarum* KSBT56 strain. The fatty acid biosynthesis (e.g., fab gene) or quorum sensing (e.g., luxS gene) of bacterial strain must be associated with the tolerance to acidic environment (Defoirdt et al. 2004; Hamon et al. 2014). Adhesion is the process of reversible accumulation of bacterial cells belonging to autoaggregation or coaggregation. Probiotics must encode aggregation promoting factor (e.g., Apf), FbpA protein or adh gene to colonize and exert antimicrobial (e.g., albE) and immune-modulatory (e.g., slpA) substances (Papadimitriou et al. 2015). *Bacillus licheniformis*, *Bacillus mycoides*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus amyloliquefaciens*, *Bacillus endophyticus*, *Bacillus halodurans*, *Bacillus paralicheniformis* and *Bacillus methylotrophicus* contain class II lantibipeptide that can be identified by the expression of *lanM* gene (Zhao and Kuipers 2016). The genome mining study revealed expression of sublacin 168 and other putative gene clusters of glycoscins in *B. thuringiensis*, *B. cereus*, *Bacillus weihenstephanensis*, *Bacillus lehensis*, *Bacillus sp.*, *Geobacillus sp.* and *Paenibacillus sp.* (Zhao and Kuipers 2016). *B. thuringiensis*, *B. cereus* and *Bacillus sp.* BH072 were reported to contain gene clusters of transmembrane protein colicins that depolarize the cytoplasm membrane of pathogen leading to dissipation of

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**Table 1. Mode of action of aquaculture probiotics.**

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of inhibitory substances to the pathogen</td>
<td>Gatesoupe 1999</td>
</tr>
<tr>
<td>Competition for adhesion sites and nutrients</td>
<td>Fuller 1989</td>
</tr>
<tr>
<td>Improvement in nutrient digestion</td>
<td>Afrilasari et al. 2017</td>
</tr>
<tr>
<td>Stimulation of innate immunity</td>
<td>Kim and Austin 2006</td>
</tr>
<tr>
<td>Elevates phagocytic activity</td>
<td>Butprom et al. 2013</td>
</tr>
<tr>
<td>Growth promotion</td>
<td>Falaye et al. 2016</td>
</tr>
<tr>
<td>Influence on water quality</td>
<td>Crab et al. 2010</td>
</tr>
<tr>
<td>Stress tolerance</td>
<td>Fuller 1989</td>
</tr>
<tr>
<td>Interference in quorum sensing</td>
<td>Defoirdt et al. 2004</td>
</tr>
<tr>
<td>Antifungal activity</td>
<td>De et al. 2014</td>
</tr>
<tr>
<td>Antiviral activity</td>
<td>Sahu et al. 2008</td>
</tr>
<tr>
<td>Protection against infection</td>
<td>Gram et al. 1999</td>
</tr>
<tr>
<td>Production of extracellular enzymes</td>
<td>Irianto and Austin 2002</td>
</tr>
<tr>
<td>Production of vitamins</td>
<td>Balcazar et al. 2006</td>
</tr>
<tr>
<td>Production of siderophores</td>
<td>De et al. 2014</td>
</tr>
<tr>
<td>Improvement of host reproduction rate</td>
<td>Ghosh et al. 2004</td>
</tr>
<tr>
<td>Improvement of haematological profile</td>
<td>Ayoola et al. 2013</td>
</tr>
<tr>
<td>Bioremediation</td>
<td>Devaraja et al. 2013</td>
</tr>
</tbody>
</table>
Cultivation of Clarias batrachus using probiotics

Table 2. Feed probiotics used in aquaculture.

<table>
<thead>
<tr>
<th>Host</th>
<th>Probiotic</th>
<th>Effect on host</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicentrarchus labrax (European sea bass)</td>
<td>Debaryomyces hansenii, Saccharomyces cerevisiae</td>
<td>Enhanced growth performance and feed efficiency</td>
<td>Tovar et al. 2002</td>
</tr>
<tr>
<td>Epinephelus coioides (Grouper)</td>
<td>Bacillus pumilus SE5 and Bacillus clausii DES</td>
<td>Improved growth performance and immune responses</td>
<td>Sun et al. 2010</td>
</tr>
<tr>
<td>Ictalurus punctatus (Channel catfish)</td>
<td>Bacillus strain</td>
<td>Prevented enteric septicaemia of catfish (ESC)</td>
<td>Ran et al. 2012</td>
</tr>
<tr>
<td>Labeo rohita (Rohu)</td>
<td>Bacillus circulans</td>
<td>Improved growth performance and feed efficiency</td>
<td>Ghosh et al. 2004</td>
</tr>
<tr>
<td>Macrobrachium rosenbergii (Prawn)</td>
<td>Bacillus subtilis</td>
<td>Enhanced growth and survivability against pathogenic Aeromonas hydrophila</td>
<td>Keysami and Mohammadpour 2013</td>
</tr>
<tr>
<td>Oncorhynchus mykiss (Rainbow trout)</td>
<td>Lactobacillus rhamnosus JCM 1136</td>
<td>Stimulated immune response</td>
<td>Panigrahi et al. 2005</td>
</tr>
<tr>
<td>Oncorhynchus mykiss (Rainbow trout)</td>
<td>Carnobacterium maltaromaticum B26, Carnobacterium divergens B33</td>
<td>Enhanced the cellular and humoral immune responses</td>
<td>Kim and Austin 2006</td>
</tr>
<tr>
<td>Oncorhynchus mykiss (Rainbow trout)</td>
<td>Bacillus subtilis AB1</td>
<td>Controlled Aeromonas infection</td>
<td>Newaj-Fyzul et al. 2007</td>
</tr>
<tr>
<td>Oncorhynchus mykiss (Rainbow trout)</td>
<td>Aeromonas hydrophila, Vibrio fluvialis, Carnobacterium sp.</td>
<td>Enhanced growth performance and feed efficiency</td>
<td>Irianto and Austin 2002</td>
</tr>
<tr>
<td>Oncorhynchus mykiss (Rainbow trout)</td>
<td>Lactobacillus rhamnosus (ATCC 53103)</td>
<td>Stimulated immune responses</td>
<td>Nikoskelainen et al. 2003</td>
</tr>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>Bacillus subtilis (ATCC 6633), Lactobacillus acidophilus</td>
<td>Stimulated the gut immune system; enhanced the immune and health status; increased the survival rate and body-weight gain</td>
<td>Aly et al. 2008</td>
</tr>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>Streptococcus faecium, Lactobacillus acidophilus, Saccharomyces cerevisiae</td>
<td>Increased growth, digestibility and feed conversion ratio</td>
<td>Lara-Flores et al. 2003</td>
</tr>
<tr>
<td>Penaeus monodon (Asian tiger shrimp)</td>
<td>Bacillus sp. 511</td>
<td>Enhanced growth performance and feed efficiency</td>
<td>Rengpipat et al. 1998</td>
</tr>
<tr>
<td>Salmo salar (Atlantic salmon)</td>
<td>Carnobacterium divergens 6251</td>
<td>Inhibited Aeromonas salmonica and Vibrio anguillarum-induced pathogenicity</td>
<td>Ringo et al. 2007</td>
</tr>
<tr>
<td>Salmo salar (Atlantic salmon)</td>
<td>Carnobacterium sp.</td>
<td>Inhibited Aeromonas salmonica, Vibrio ordali, Yersinia ruckeri and reduced disease outbreak</td>
<td>Robertson et al. 2000</td>
</tr>
</tbody>
</table>

The nutritional profile of C. batrachus contains easily digestible high-grade protein (16.26 g/100 g), iron (2.20 mg/100 g), minerals, good cholesterol and polyunsaturated fatty acids (ICAR-CIFA). It also contains a rich source of vitamin A (6.03 IU/100 g), vitamin D (44.73 IU/100 g) and essential amino acids (ICAR-CIFA; Mohanty et al. 2014).

The body, generally grayish-black, is cylindrical and tapers towards the caudal peduncle. C. batrachus attains a standard length of 225–300 mm. However, in India it is found to be around 183.1 mm in length as an average (Ng and Kottelat 2008).

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C. batrachus can be found in both fresh and brackish water of Sri Lanka, India, Pakistan, Bangladesh, China, Burma, Malaya, Singapore, Philippines, Borneo, Java, and Thailand (Talwar and Jhingran 1991). They can be found in a variety of habitats, most commonly in muddy or swampy low-land field and rice fields. The major constraint in the cultivation of C. batrachus in natural resources is the non-availability of quality seeds. The scarcity of seeds arises from various factors like: indiscriminate use of pesticides in paddy fields, industrial effluents, diminution...
of breeding area due to siltation, intermittent periods of drought and illicit fishing of juveniles and brood fishes (Dhara and Saha 2013). The exotic catfish *C. gariepinus* that has morphological resemblance to indigenous *C. batrachus* is frequently misled by some dishonest traders (Khedkar et al. 2015). These have threatened the mere existence of this indigenous catfish. Therefore, it becomes crucial to carry out breeding and rearing of *C. batrachus* to meet the need of the society. As the species is sold only in living condition and cannot be transported over long distances, a culturing in semi-intensive manner becomes necessary. Thus, cultivation in small production pond and its supply to the market in living condition would be a better practice for catfish farming.

**Probiotics in Clarias species**

The invasive alien catfish *C. gariepinus* has presently been considered as a potential threat due to its frenzied feeding behavior and hence, farming of indigenous *C. batrachus* is regaining its importance (Radhakrishnan et al. 2011). However, reports on the use of probiotics in *C. gariepinus* are available, but our knowledge is still limited so far as *C. batrachus* (Table 4) are concerned. In this regard, a protocol to screen putative probiotic strain for cultivation of *C. batrachus* can be proposed (Fig. 2).

Probiotic strains usually synthesize extracellular enzymes (e.g., proteases, amylases, lipases) and growth factors (e.g., vitamins, fatty acids, amino acids) which can stimulate the appetite and endorse fish nutrition by the detoxification of toxic substances and breakdown of indigestible components (Irianto and Austin 2002; Balcazar et al. 2006). Consequently, nutrients are more readily absorbed when the feed is supplemented with probiotics (Afrilasari et al. 2017). The use of *Lactobacillus acidophilus* with a diet for 12 weeks has exhibited improved specific growth rate (SGR), relative growth rate (RGR), protein efficiency ratio (PER), feed conversion ratio (FCR), haematological parameter and significantly (p < 0.05) higher survival rate (SR) in *C. gariepinus* fingerlings (Ige 2013).

Banerjee and co-workers (2015) isolated an extracellular enzyme-producing bacterial strain *Bacillus licheniformis* from *C. batrachus*. Dey et al. (2016) obtained an extracellular enzyme-producing autochthonous gut bacteria *B. cereus* HG01 (KR809412) from *C. batrachus*. Ayo Olalusi et
Cultivation of Clarias batrachus using probiotics

Putra et al. (2017) also reported to achieve better growth performance and feed utilization efficiency with non-probiotic diet. Probiotic Bacillus aryabhattai than those with pathogenic Salmonella typhi (Hapsari 2016). Putra et al. (2017) also reported to achieve better growth performance and feed utilization efficiency in African catfish C. gariepinus using biofloc technology infused with the Bacillus probiotic.

Probiotic microorganisms often exert bactericidal or bacteriostatic substances to restrict the propagation of pathogenic bacteria (Sahu et al. 2008). Kato et al. (2016) have conducted a study to isolate and identify probiotic bacteria from the surface of the African catfish C. gariepinus. Among all the isolates, Lactococcus sp. and Lactobacillus sp. have shown potential antimicrobial activity against gram negative bacteria Citrobacter, E. coli, Klebsiella, Proteus, Pseudomonas and Salmonella (Ogunshe and Olabode 2009). Fortified diet enriched with L. plantarum enhanced growth, weight gain and FCR of cultured C. gariepinus fingerlings (Falaye et al. 2016). A parallel study with C. gariepinus showed increased growth performance, FCR, PER, protein productive value and energy retention when the fish feed was supplemented with a commercial probiotic strain of Bacillus (El-Haroun 2007). The organism germinated in the intestine and synthesized digestive enzymes amylase, protease and lipase which in turn contributed improved feed efficiency.

Probiotics often exert immunomodulating substances to stimulate immune response against pathogenic invasion (De et al. 2014). Vibrio anguillarum, Vibrio alginolyticus and Aeromonas hydrophila were reported to cause pathogenicity in C. batrachus (Ahmed et al. 2012). Dahiya et al. (2012) successfully experimented with C. batrachus fingerlings, treating them with probiotics that resulted remarkable increase of immunity and hemoglobin level of the catfish. L. plantarum C014 infused (10⁷ cfu/g) diet improved innate immune response and disease resistance ability of hybrid catfish. The probiotic-supplemented diet elevated

**Table 3. Examples of water probiotics used in aquaculture.**

<table>
<thead>
<tr>
<th>Host</th>
<th>Probiotic</th>
<th>Effect on host</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litopenaeus vannomei (Pacific white shrimp)</td>
<td>Bacillus sp. and Lactobacillus</td>
<td>Improved the environmental quality of the sediment and water in ponds with closed recirculation systems</td>
<td>Paiva-Maia et al. 2013</td>
</tr>
<tr>
<td>Oncorhynchus mykiss (Rainbow trout)</td>
<td>Pseudomonas fluorescens AH2</td>
<td>Increased survival rate against pathogenic Vibrio anguillarum</td>
<td>Gram et al. 1999</td>
</tr>
<tr>
<td>Penaeus monodon (Asian tiger shrimp)</td>
<td>Bacillus sp.</td>
<td>Improved growth and survival rate, maintained water quality</td>
<td>Dalmin et al. 2001</td>
</tr>
<tr>
<td>Scophthalmus maximus (Turbot larva)</td>
<td>Bacillus pumilus, B. licheniformis and B. subtilis</td>
<td>Reduced total ammonia nitrogen (TAN); improved growth and survival rate</td>
<td>Devaraja et al. 2013</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>Lactic acid bacteria</td>
<td>Increased survival rate against vibriosis</td>
<td>Gatesoupe 1994</td>
</tr>
<tr>
<td>Roseobacter sp.</td>
<td>L. plantarum</td>
<td>Improved survival rate</td>
<td>Hjelm et al. 2004</td>
</tr>
</tbody>
</table>

al. (2014) showed that viable feed-probiotics administered to C. gariepinus increased the hematological parameter and digestive enzyme (amylase and lipase) activity of the catfish within acceptable range. Probiotic L. plantarum infused diet considerably enhanced hematological parameters, carcass protein and mineral composition of African catfish C. gariepinus (Nwanna and Tope-Jegede 2016).

A higher level of immunity was also noted while challenging with pathogenic Salmonella typhi than those with non-probiotic diet. Probiotic Bacillus aryabhattai KP784311, B. flexus KR809411, B. cereus KR809412 encapsulated chironomid midge larvae significantly (p < 0.05) increased specific growth rate and survivability of C. batrachus (Dey et al. 2017).

Synergistic relationships among different bacterial strains may be more effective and consistent than a single strain of probiotic (Salinas et al. 2008). L. plantarum and Pseudomonas fluorescens have a synergistic effect on each other and therefore produced higher specific growth rate and survival rate in C. gariepinus fingerlings than the control diet (Omenwa et al. 2015). Ayoola et al. (2013) reported that administering a mixture of Lactobacillus and Bifidobacterium species in a feeding trial (90 days) enhanced feed efficiency, growth rate, survivability and nutritional quality of C. gariepinus juveniles. The hydrobiological parameters are also important in maintaining the integrity of aquatic ecosystem and have direct influence on the productivity of C. batrachus (Ganguly et al. 2017).

Biofloc technology is a sustainable method to meliorate water quality and feed utilization efficiency of aquatic animals (Crab et al. 2010). The fermented bioflocs inoculated with the bacterium B. cereus enhanced the growth and feed utilization efficiency of juvenile catfish C. gariepinus (Hapsari 2016). Putra et al. (2017) also reported to achieve better growth performance and feed utilization efficiency in African catfish C. gariepinus using biofloc technology infused with the Bacillus probiotic.
phagocytic activity, lysozyme efficacy and survival rate of hybrid catfish against *A. hydrophila* infection (Butprom et al. 2013).

Probiotic microorganisms resist the establishment of pathogen by adsorbing and colonizing the digestive tract of the host (Fuller 1989) through a process called competitive exclusion. One experiment was carried out to evaluate the effects of the probiotic bacterium *Bacillus megaterium* PTB 1.4 on the growth performance, intestinal microflora and digestive enzyme activity of *Clarias sp.* (Afrilasari et al. 2017). The results showed significantly higher (p < 0.05) SGR and increased activity of protease and amylase in fish maintained on the probiotic-supplemented diet compared to those on the control diet. Bairagi et al. (2002) isolated distinct microbial source of digestive enzymes amylase, lipase and protease from the gastrointestinal tract of *C. batrachus* that may contribute towards better feed formulations. Yakubu et al. (2016) assessed the effects

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**Figure 2.** Diagram for screening of autochthonous probiotic strain for the cultivation of *C. batrachus*. 
of commercial probiotic (Biogut) on *C. gariepinus* and observed improved growth and survival rate of the fry. Jahan et al. (2016) noticed better growth performance, SGR and proximate carcass compositions of *C. batrachus* fingerlings fed with probiotic-supplement diet compared to the control diet.

Quorum sensing is a bacterial cell-to-cell communication mechanism leading to the alternation of gene expression in response to high population density (De Almeida et al. 2016). The quorum quenching or disruption of quorum sensing is considered as potential anti-infective strategy in aquaculture (Defoirdt et al. 2004). The probiotic *Lysinibacillus sphaericus, B. amyloliquefaciens* and *B. cereus* have been reported to disintegrate acyl homoserine lactone (AHL), the quorum sensing molecule of pathogenic *A. hydrophila* by producing AHL-lactonase and thus preventing motile aeromonad septicemia (MAS) in *C. gariepinus* (Novita et al. 2015).

## Conclusion

In the era of global food crisis, aquaculture stands as a sustainable approach to restore biodiversity and ensure nutritional security (Naylor et al. 2000). Countries where a large section is battling with protein deficiency and malnutrition, consumption of *C. batrachus* may hold promising potentiality in uplifting the overall health status of the populace. To reinstate the genetic resources of *C. batrachus*, semi-intensive aquaculture practices has to be adopted. A concerted effort is the need of the hour to cultivate *C. batrachus* due to its apparent nutritional and economic significance, whereas rational selection and proper validation of probiotic is a cause of concern. The limnological properties of aquatic pond may pose a threat to the establishment of probiotics and thus affect fish health. The laboratory result with test probiotics should be in accordance with large-scale commercial implication. The preservation of probiotics maintaining the viability of the organisms is yet to be standardized. The dose-level optimization of a certain probiotic strain also needs to be carried out before commercialization.

However, different molecular technique-based approaches (polymerase chain reaction, multiplex-PCR, pulsed field gel electrophoresis, random amplified polymorphic DNA, fourier-transform infrared spectroscopy, denaturing gradient gel electrophoresis, temporal temperature gradient gel electrophoresis, fluorescence *in situ* hybridization) are now being increasingly used for the analysis of GI microflora to screen autochthonous

### Table 4. Application of probiotics in *Clarias* species.

<table>
<thead>
<tr>
<th>Host</th>
<th>Probiotic</th>
<th>Effect on host</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clarias batrachus</em></td>
<td><em>Lysinibacillus sphaericus</em></td>
<td>Inhibited <em>Vibrio harveyi</em> infection</td>
<td>Ganguly et al. 2018</td>
</tr>
<tr>
<td><em>Clarias batrachus</em> (Asian catfish)</td>
<td><em>Lactobacillus sporogenes, Saccharomyces boulardii</em></td>
<td>Controlled <em>A. hydrophila</em> infection</td>
<td>Dahiya et al. 2012</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em> (African catfish)</td>
<td><em>Lactobacillus acidophilus</em></td>
<td>Enhanced haematology parameters, stimulated immunity, inhibited <em>Staphylococcus xylosus, Aeromonas hydrophila</em> gr.2 and <em>Streptococcus agalactiae</em> infection</td>
<td>Al-Dohail et al. 2011</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em> (African catfish)</td>
<td><em>Bacillus thuringiensis</em></td>
<td>Enhanced of cellular non-specific immune response against <em>A. hydrophila</em> infection</td>
<td>Reneshwary et al. 2011</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em> (African catfish)</td>
<td><em>Lactobacillus acidophilus</em></td>
<td>Improved fish health, enhanced haematologica l parameters</td>
<td>Olayinka and Afolabi 2013</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em> hybrid (MCF 1× QCF 2) (Egyptian African catfish)</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Increased body weight and growth rate</td>
<td>Essa et al. 2011</td>
</tr>
<tr>
<td><em>Clarias orientalis</em> (Catfish)</td>
<td><em>Lactobacillus sp.</em></td>
<td>Increased growth and survival rate, inhibited <em>Aeromonas</em> and <em>Vibrio</em> sp.</td>
<td>Dhanasekaran et al. 2008</td>
</tr>
</tbody>
</table>
probiotics (Kim et al. 2007). The ‘omics’ studies may also provide a potential opportunity to obtain probiotic microorganisms avoiding traditional cultivation methods. The use of probiotics to potentiate the benefits of C. batrachus stand necessary and its application is both empirical and scientific. A futuroistic approach with probiotics maintaining ecologically sound management practices has to be adopted to bring about socio-economic upliftment in Asian countries which are the native place of C. batrachus.

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