Review of literature

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Probiotics establish symbiotically within the host digestive tract. They can increase the growth and vigour of the host species by successful physiological and nutritional modulation. In aquaculture, probiotics have become important biological entity for pursuing increased rate of production. The knowledge on aquaculture probiotics are flourishing and the series of important findings regarding the current research are being reviewed.

Aquaculture probiotics

Aquaculture probiotics must be able to colonize the gastrointestinal tract of aquatic species which is constantly been affected by the flow of water passing through the digestive tract (Gatesoupe 1999). Generally, commercial aquaculture probiotics are available both in dietary and liquid forms. Fuller (1989) emphasized on the dietary application of probiotics to the host in order to improve the gastrointestinal microbiota. The dietary probiotics (Table 1) usually consist of spore-forming microorganisms. They can be directly incorporated into the basal feed with the help of a binder. The basal feed often possesses vitamins and other nutritional additives (Bandyopadhyay and Das Mohapatra 2009).

Alternatively, probiotics and the other ingredients can be mixed with sterile water for brewing at 27-32 °C for 16 to 18 h with continuous agitation (Sahu et al. 2008). Moriarity (1998) insisted the requirement of liquid probiotics in aquaculture beside the traditional feed probiotic (Gatesoupe 1999). The commercial liquid probiotics (Table 2) are applied directly to the culture water in the morning and evening avoiding sunlight due to their hygroscopic nature (Sahu et al. 2008). The immersion method of treating fish culture in a probiotic-rich container for a certain period of time on a regular basis to enhance the

host immunity is also gaining increased attention (Feliatra et al. 2018). Probiotics have been found to play a significant role in the sustainable development of aquaculture through different approaches (Table 3).

Table 1: Examples of feed probiotics used in aquaculture.

Probiotics	Host species	Effect on host	References
Bacillus toyoi	Japanese eel	Enhanced growth rate; reduced mortality against pathogenic *Edwardsiella* sp.	Kozasa 1986
Bacillus IP58 32	Rotifers	Enriched nutritional content	Gatesoupe 1993
Bacillus sp. S11	*Penaeus monodon* (Asian tiger shrimp)	Improved growth performance; enhanced feed efficiency	Rengpipat et al. 1998
Carnobacterium sp.	*Salmo salar* (Atlantic salmon)	Reduced disease outbreak by inhibiting *Aeromonas salmonicida*, *Vibrio ordalii* and *Yersinia ruckeri*.	Robertson et al. 2000
Aeromonas hydrophila, Vibrio fluvialis, Carnobacterium sp.	*Oncorhynchus mykiss* (Rainbow trout)	Enhanced growth rate; augment feed efficiency	Irianto and Austin 2002
Debaryomyces hansenii, Saccharomyces cerevisiae	*Dicentrarchus labrax* (European sea bass)	Improved growth performance; enhanced feed efficiency	Tovar et al. 2002
Streptococcus faecium, Lactobacillus acidophilus, S. cerevisiae	*Oreochromis niloticus* (Nile tilapia)	Stimulated growth and nutrient digestibility; reduced feed conversion ratio	Lara-Flores et al. 2003
Lactobacillus rhamnosus	*Oncorhynchus mykiss* (Rainbow trout)	Stimulated immune responses	Nikoskelainen et al. 2003
Bacillus circulans	*Labeo rohita* (Rohu)	Enhanced growth performance; increased feed utilization efficiency	Ghosh et al. 2004
Lactobacillus rhamnosus	*Oncorhynchus mykiss* (Rainbow trout)	Enhanced immune response	Panigrahi et al. 2005
Lactococcus lactis , Lactobacillus sakei, Leuconostoc mesenteroides	*Oncorhynchus mykiss* (Rainbow trout)	Stimulated nonspecific immune response; enhanced phagocytosis	Balcazar et al. 2006
Carnobacterium, maltaromaticum, Carnobacterium divergens	*Oncorhynchus mykiss* (Rainbow trout)	Stimulated humoral and cell-mediated immune responses	Kim and Austin 2006
Carnobacterium divergens	Atlantic salmon (*Salmo salar* L.)	Showed antagonism against pathogen	Ringo et al. 2007
Bacillus subtilis	*Oncorhynchus mykiss*	Inhibited *Aeromonas* infection	Newaj-Fyzul et al. 2007
B. subtilis and *L. acidophilus*	*Oreochromis niloticus* (Nile tilapia)	Stimulated immune system; developed the health status; increased the survival rate and body-weight gain	Aly et al. 2008

Micrococcus luteus, *Pseudomonas* sp.	*Oreochromis niloticus* (Nile tilapia)	Enhanced growth performance, survival rate and feed utilization efficiency; reduced mortality against *Aeromonas hydrophila*	Abd el-Rhman et al. 2009
Bacillus pumilus and *Bacillus clausii*	*Epinephelus coioides* (Grouper)	Increased growth performance; enhanced immune responses	Sun et al. 2010
Bacillus sp.	*Ictalurus punctatus* (Channel catfish)	Inhibited enteric septicaemia of catfish (ESC)	Ran et al. 2012
B. subtilis	*Macrobrachium rosenbergii* (Prawn)	Enhanced growth and survival rate against pathogenic *Aeromonas hydrophila*	Keysami and Mohammadpour 2013
B. pumilus	*Labeo rohita*	Enhanced the haematological profile	Rajikkannu et al. 2015
S. cerevisiae, *B. subtilis* and *B. cereus*	*Ctenopharyngodon idella* (Grass Carp)	Improved the growth performance and healthy status of fish	Toutou et al. 2016
Bacillus stratosphericus, *Phaeobacter daeponensis*	*Haliotis diversicolor* (small abalone)	Increased health and nutrient status	Zhao et al. 2018
Bacillus velezensis	*Ictalurus punctatus*	Promoted catfish growth and improved pond water quality	Thurlow et al. 2018
Bacillus licheniformis, *B. flexus*	*Litopenamei vannamei*	Enhanced growth, survivability, enzymatic activity and immune response	Cai et al. 2019

Table 2: Examples of water probiotics used in aquaculture.

Probiotic	Host	Effect on host	References
Lactic acid bacteria	*Scophthalmus maximus* (Turbot larva)	Decreased mortality against vibriosis	Gatesoupe 1994
Bacillus sp.	Channel catfish	Enhanced production rate; increased survival ratio	Queiroz and Boyd (1998)
Pseudomonas fluorescens AH2	*Oncorhynchus mykiss* (Rainbow trout)	Improved survival rate against pathogenic *Vibrio anguillarum*	Gram et al. 1999
Bacillus sp.	*Penaeus monodon* (Asian tiger shrimp)	Enhanced growth and survival rate, maintained water quality	Dalmin et al. 2001
Roseobacter sp.	*Scophthalmus maximus* (Turbot larvae)	Decreased mortality	Hjelm et al. 2004
Bacillus sp., *S. cerevisiae*, *Nitrosomonas* sp.	*Penaeus vannamei* (White shrimp)	Reduced concentrations of nitrogenous components. Enhanced productivity	Wang et al. 2005
Lactobacillus acidophilus and *Saccharomyces cerevisiae*	*Cyprinus carpio*	Enhanced growth rate, survivability; improved feed conversion ratio	Ramakrishnan et al. 2008
Lactobacillus	*Ctenopharyngodon idella*	Increased survivability; enhanced nutrient digestion	Sheeja et al. 2003
Bacillus sp., *Lactobacillus*	*Litopenaeus vannamei* (Pacific white shrimp)	Improved the pond ecosystem	Paiva-Maia et al. 2013
Bacillus pumilus, *B. licheniformis*, *B. subtilis*	*Penaeus monodon* (Asian tiger shrimp)	Decreased total ammonia nitrogen (TAN); enhanced growth and survivability	Devaraja et al. 2013
Ettlia sp.	*Danio rerio*	Improved water quality through reduction of nitrogenous compounds	Chun et al. 2018
L. acidophilus, L. plantarum, L. rhamnosus	*Hippocampus erectus* (seahorse)	Prevented enteritis	Lin et al. 2019

Table 3: Mode of action of aquaculture probiotics.

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

Molecular identification techniques for probiotics

Molecular tracking of probiotic markers (Fig. 1) were recently been prioritized over traditional approaches to properly validate putative probionts (Papadimitriou et al. 2015). The probiotic may express bile salt-regulatory (*e.g., bsh*), adhesion (*e.g., adh*), fatty acid biosynthesis (*e.g., fab*) or quorum sensing (*e.g., luxS*) genes, aggregation promoting factors (*e.g.,* Apf), molecular chaperones (*e.g., DnaK, GroES*) or DNA repair proteins (*e.g., uvrB*) in order to survive and colonize the host intestine (Defoirdt et al. 2004; Hamon et al. 2014). Tripathy et al. (2014) identified several probiotic markers (bile salt hydrolase, fibronectin binding protein and mucus binding protein) in *Lactobacillus plantarum* KSBT56 strain.

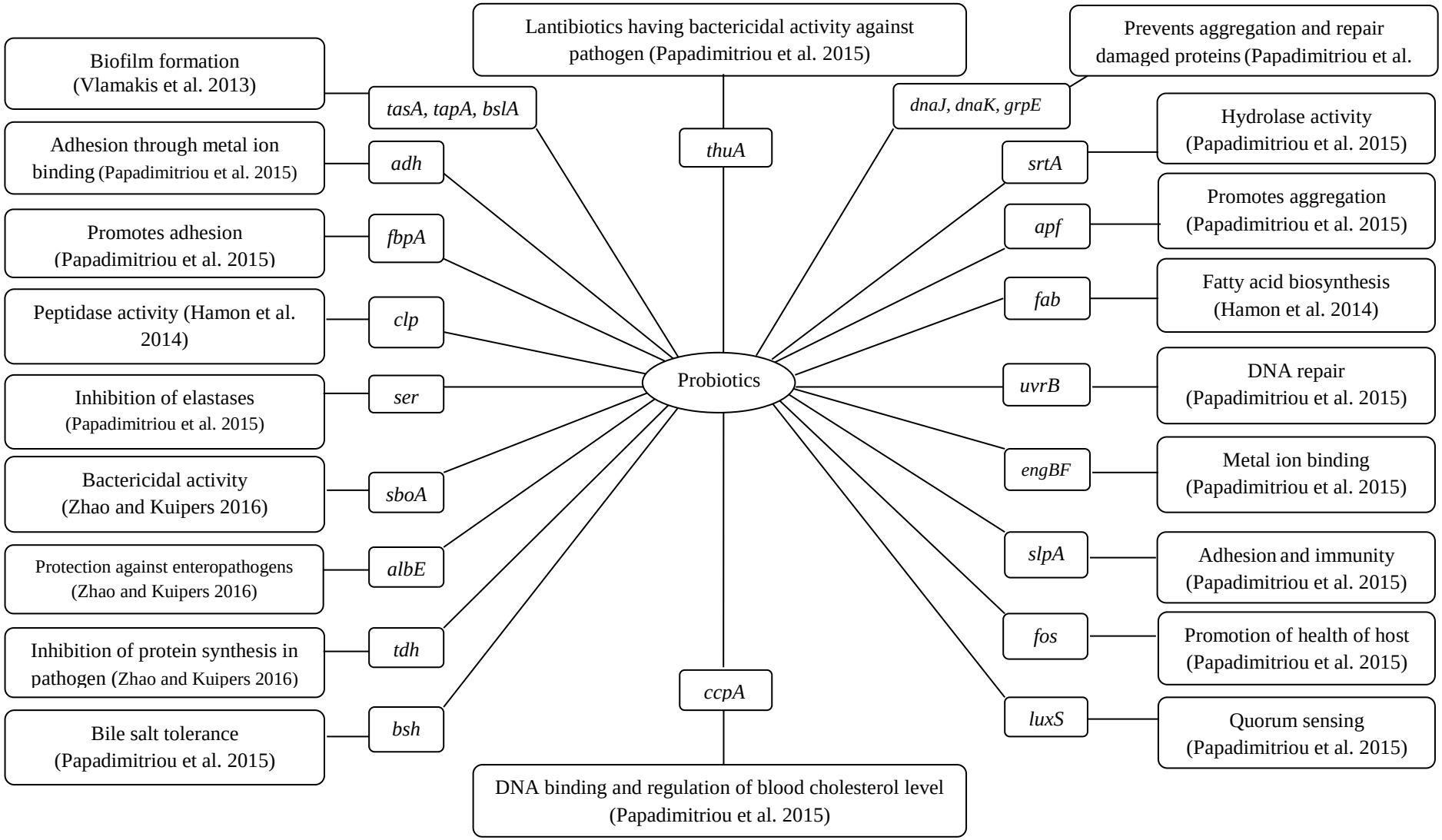

Fig. 1: Genes related to potential probiotic properties.

Probiotics may also contain immune-modulators (*e.g.,* Sublancin 168, *slpA*) or antimicrobial substances (*e.g., albE*) (Papadimitriou et al. 2015). Zhao and Kuipers (2016) identified the transmembrane protein colicins (depolarize the cytoplasm membrane of pathogen) in *B. thuringiensis, B. cereus* and *Bacillus* sp. BH072 strains. Probiotic isolates (*Bacillus cereus*, *B. mycoides*, *B. thuringiensis*, *B. licheniformis*, *B. amyloliquefaciens, B. halodurans*, *B. endophyticus*, *B. paralicheniformis* and *B. methylotrophicus*) may exert class II lanthipeptide that can be identified through the expression of *lanM* gene (Zhao and Kuipers 2016). The formation of biofilm through the secretion of extracellular matrix proteins is another aspect of probiotics. Majed et al. (2016) revealed the presence of biofilm forming genes (*e.g., bslA, tapA*) in *B. cereus* and *B. thuringiensis*. The further identification of molecular markers including housekeeping genes can enrich the understanding about the probable mode of action of probiotics.

Microbial infections in *Clarias* species

C. batrachus is one of the most desired 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 pathogenic infections are one of the major causes of productivity constraint in *Clarias* sp. (Table 4). In aquaculture, prevention and control of aquatic diseases by chemical additives or antibiotics may generate antibiotic-resistant bacteria and thus creates a serious concern to public health (FAO 2006). There are many reports regarding a sharp decrease in productivity due to abrupt use of anti-microbial drugs (Alcaide et al. 2005).

Table 4: Common diseases of *Clarias* sp.

Type	Etiological agent	Host	Effect on host	References
Bacterial	*Edwardsiella ictaluri*	*C. batrachus*	Bacterial infection	Kasornchandra et al. 1987
	A. hydrophila	*C. batrachus*	Ulcerative lesions on head, skin.	Llobrera and Gacutan 1987
	A. hydrophila	*C. gariepinus*	Ulcerative lesions on body surface and kidney	Rahman and Chowdhury 1999
	Flexibacter columnaris	*C. gariepinus*	Columnaris disease	Ikpi and Offem 2011
	Providencia sp.	*C. batrachus*	Ulcerative lesions on skin	Thomas et al. 2013
	Pseudomonas aeruginosa	*C. gariepinus*	Immunosuppression, tissue degeneration and necrosis	Magdy et al.2014
	E. ictaluri	Hybrid catfish	Enteric septicaemia	Suanyuk et al. 2014
	Edwardsiella tarda	*C. gariepinus*	Edwardsiellosis	Abraham et al. 2015
	Aeromonas hydrophila, A. caviae, A. sobria, A. jandaei, A. rivuli, A. aquariorum, A. fluvialis	*C. batrachus*	Bacterial septicaemia	Paul et al. 2015
Viral	*Ictalurid Herpes virus* 1	*Clarias* sp.	CCVD (channel catfish virus diseases).	Boon and Huisman 1996
Fungal	*Tricophyton, Rhizopus, Saprolegnia, Mucor, Penicillium, Microsporum* and *Alternaria*	*C. gariepinus*	Infection in eggs	Melaku et al. 2017
	Mucor, Aspergillus, Trichophyton, and *Penicillium*	*C. gariepinus*	Localized surface infection	Atawodi et al.2017
Parasitic diseases	*Trichodina* sp., *Epistylis* sp. and *Gyrodactylus* sp.	*C. gariepinus*	Ectoparasitic Diseases	Abo-Esa 2008
	Trichodina matsu, T. magna, T. maritinkae and *T. sangwala*	*C. gariepinus*	Ectoparasitic disease on gill and skin	El-Tantawy and El-Sherbiny 2010
	Saprolegnia, Icthyophonus, Candida albicans, Achlya and *Aspergillus fumigatus*	*C. gariepinus*	Parasitic infection on skin	Nwabueze 2012
	Djombangia penetrans, Lytocestus indicus, L. birmanicus, L. parvulus and *Bovienia serialis*	*C. batrachus*	Parasitic infestation	Laboni et al. 2012
	Gyrodactylus alekosi sp. and *G. rysavyi*	*C. gariepinus*	Parasitic infections	Přikrylová et al. 2012
	Ichthyophthirius multifiliis , Trichodina sp., *Epistylis* sp., *Gyrodactylus* sp.	*C. gariepinus*	Ectoparasitic infection	Ghoneim et al. 2015
	Procamallanus sp., *Polynchobothrium clarias, Diphyllobothrium latum, D. plerocercoid, Diplostomum spathaceum, Acanthocephalan* sp.	*C. gariepinus*	Endoparasitic disease in intestine	Balogun and Solomon 2015
	Dactylogyrus, Gyrodactylus, Trichodina and *Chilodinella*	*C. gariepinus*	Ectoparasitic Diseases	Gado et al. 2017

Probiotics in *Clarias* species

Earlier studies on *C. batrachus* were majorly contributed on taxonomic status, distribution pattern, habitat range, physiological features and feeding habits. Jayaram (1981) reported that *C. batrachus* was the most predominant out of seven species of *Clarias* found in Indian subcontinent. The pollutant intoxication in *C. batrachus* through culture pond water was studied by Dhilon and Gupta (1982). The study of enzymatic activities correlated to the dietary habits of *C. batrachus* was conducted by Mukhopadhyay (1977). The effect of feeding frequency on the growth and distribution of *C. batrachus* was evaluated by Mollah and Nurullah (1988). The effect of toxicity of sodium arsenite on hematological profile was investigated by Kumar and Banerjee (2016). The breeding pattern of *C. batrachus* under intensive cultivation was analyzed by Tripathi (1996). Datta Munshi (1961) thoroughly studied the additional respiratory organs of *Clarias batrachus.* Sarma et al. (2012) assessed the growth, biochemical composition and rate of survivability of *C. batrachus* under severe saline condition. The screening and application of autochthonous probiotic for the cultivation of indigenous *Clarias batrachus* is in major focus worldwide. However, reports on the use of probiotics in *C. gariepinus* are much available, but the knowledge is still limited so far as *C. batrachus* (Table 5) are concerned. The necessity of sustainable aquaculture has promoted research on the use of probiotics for the cultivation of *Clarias* species through different approaches:

Improvement in nutrient digestion

Probiotic isolates often secrete extracellular enzymes (e.g., amylases, proteases, lipases) or growth factors (e.g., vitamins, siderophores, fatty acids, amino acids) which can digest indigestible food components more efficiently and promote fish nutrition (Irianto and

Austin 2002; Balcazar et al. 2006). Al-Dohail et al. (2009) observed enhanced growth, survivability and feed utilization efficiency in *C. gariepinus* by supplementing the feed with *Lactobacillus acidophilus.* Banerjee et al. (2015) isolated potential enzyme-synthesizing bacterial isolate *Bacillus licheniformis* from adult *C. batrachus*. Olayinka and Afolabi (2013) applied *Lactobacillus acidophilus* for the cultivation of *C. gariepinus* and observed improved fish health and haematological parameters. Dey et al. (2016a) isolated *B. cereus* HG01 (KR809412) strain from adult *C. batrachus*. The organism was capable of synthesizing extracellular digestive enzymes. The substantial application of viable feed-probiotics can enhance haematological profile and enzymatic activity *C. gariepinus* (Ayo Olalusi et al. 2014). Falaye et al. (2016) reported enhanced growth, weight gain and FCR in *C. gariepinus* fingerlings through applying fortified diet infused with *L. plantarum*. Dey et al. (2016b) isolated autochthonous putative probiotic strain *Bacillus aryabhattai* KP784311 from the foregut of adult *C. batrachus* and obtained better growth performance in *C. batrachus* juvenile by encapsulating the probiotic with chironomid larvae. Bairagi et al. (2002) isolated enzyme (protease, amylase and lipase) producing distinct microflora from the intestinal tract of *C. batrachus* that may further contribute better feed formulations. El-Haroun (2007) used a commercial strain of *Bacillus* to obtain better growth performance, protein efficiency ratio, protein productive value and decreased feed conversion ratio in *C. gariepinus*. The probiotic isolates proliferated in the digestive tract and synthesized amylase, protease and lipase that have resulted increased feed utilization efficiency.

The consortium of probiotics may be more effective and consistent than a single strain due to their synergistic nature (Salinas et al. 2008). Omenwa et al. (2015) showed

the administration of *Lactobacillus plantarum* and *Pseudomonas fluorescens* in *C. gariepinus* fingerlings can endorse fish nutrition, survivability and increased specific growth rate. Ayoola et al. (2013) applied a consortium of *Lactobacillus* sp. and *Bifidobacterium* sp. for 90 days to *C. gariepinus* juveniles and reported enhanced feed efficiency, survivability and nutritional quality than the control fed fish.

Improvement of culture water

Biofloc system is a cost-effective technology to monitor waste removal, decompose undesirable substances, reduce water pollution, and increase productivity in aquaculture ponds (Crab et al. 2010). Hapsari (2016) utilized fermented bioflocs infused with the probiotic *B. cereus* to enhance growth and feed utilization efficiency of *C. gariepinus* juveniles. Putra et al. (2017) also observed increased growth performance and nutrient utilization ability in *C. gariepinus* using *Bacillus* sp. as probiotic supplement through biofloc technology. The application of *Lactobacillus* has improved both the culture condition and growth of *C. gariepinus* (Aderolu et al. 2013).

Production of inhibitory substances against pathogen

Probiotic microorganisms may inhibit fish pathogens by producing wide-spectrum of bactericidal or bacteriostatic chemical substances (e.g., siderophores, bacteriocins, enzymes etc.). Ogunshe and Olabode (2009) isolated *Lactobacillus plantarum* LbOGI and *Lactobacillus fermentum* LbFF4 strains from *C. gariepinus*, and observed antibacterial activity against pathogenic *Salmonella*, *Klebsiella*, *E. coli*, *Citrobacter*, *Proteus* and *Pseudomonas*. Strains of *Lactococcus* and *Lactobacillus*, isolated from the surface of *C. gariepinus* executed significant antimicrobial activity against aquaculture pathogens (Kato et al. 2016).

Stimulation of immunity

Probiotics often exert signaling molecules to stimulate humoral or cellular immune response against pathogenic invasion (De et al. 2014). Aquaculture pathogen *Vibrio harveyi, V. anguillarum* or *Aeromonas hydrophila* cause significant mortality in *C. batrachus* worldwide (Afrilasari et al. 2017). Dahiya et al. (2012a) applied probiotic *Lactobacillus sporogenes* and *Saccharomyces boulardii* to *C. batrachus* fingerlings against pathogenic *Aeromonas hydrophila* and *Micrococcus sp.* and observed increased level of immunity and haematological profile in cat fish. Butprom et al. (2013) also have reported enhanced immune response, disease resistance ability and survivability in hybrid catfish against *A. hydrophila* infection by supplementing the feed with *Lactobacillus plantarum* C014. Nwanna and Tope-Jegede (2016) evaluated the effects of probiotic *Lactobacillus plantarum* on the immune response of *C. gariepinus* by challenging with pathogenic *Salmonella typhi.* The result indicated that fish fed on probiotic supplemented feed (10^3-10^5 cfu/g) significantly enhanced the carcass protein, blood profile and immunity in experimental fish.

Competition for adhesion sites and nutrients

Probiotic microorganisms confer protection against pathogens by limiting nutritional resources through the process of competitive exclusion by adsorbing and colonizing the intestinal tract of the host (Fuller 1989). Afrilasari et al. (2017) evaluated the effects of *Bacillus megaterium* PTB 1.4 on the growth performance, gastrointestinal microflora and enzymatic activity of *Clarias sp.* and obtained significantly higher ($p<0.05$) specific growth rate and enzymatic activity. Yakubu et al. (2016) used commercial probiotic strain as a feed supplement for *C. gariepinus* and reported increased growth and survivability.

Administration of viable probiotics resulted better growth and carcass composition in *C. batrachus* fingerlings (Jahan et al. 2016).

Table 5: Application of probiotics in *Clarias sp.*

Probiotic	Host	Effect on host	References
Lactobacillus	*Clarias orientalis* (Catfish)	Enhanced growth and survival rate; inhibited *Aeromonas* and *Vibrio* sp.	Dhanasekaran et al. 2008
Lactobacillus acidophilus	*Clarias gariepinus* (African catfish)	Enhanced haematological profile, stimulated immunity, inhibited *Staphylococcus xylosus*, *Aeromonas hydrophila* gr.2 and *Streptococcus agalactiae* infection	Al-Dohail et al. 2011
Saccharomyces cerevisiae	*Clarias gariepinus* hybrid (MCF♀ × QCF♂)	Enhanced production and growth rate	Essa et al. 2011
Bacillus thuringiensis	*Clarias gariepinus*	Enhanced non-specific immune response against *A. hydrophila* infection	Reneshwary et al. 2011
Lactobacillus sporogenes, Saccharomyces boulardii	*Clarias batrachus* (Asian catfish)	Decreased *A. hydrophila* infection	Dahiya et al. 2012b
Nitromonas, Azospirillum, Rhodococcus, Pseudomonas, Bacillus licheniformis, Bacillus megaterium, Desulfovibrio desulfuricans, Chromatium, Chlorobium, Thiobacillus thioxidans, Thiobacillus ferrooxidans, Methylomonas methanica, Gluconacetobacter, Trichoderma, Schizophyllum commune and *Sclerotium*	*Clarias batrachus*	Developed haematological profile, reduced *Aeromonas hydrophila* infection	Dahiya et al. 2012c
Lactobacillus acidophilus	*C. gariepinus*	Increased SGR, RGR, PER, FCR; developed haematological profile and survivability.	Ige 2013
Lactobacillus acidophilus, Bacillus subtilis and *Lactobacillus bulgaricus*	*Clarias gariepinus*	Improved growth and survivability	Dennis and Uchenna 2016
Lactobacillus plantarum	*C. gariepinus*	Enhanced carcass protein and mineral composition; increased haematological profile.	Nwanna and Tope-Jegede 2016
Bacillus aryabhattai, *B. cereus* and *B. flexus*	*C. batrachus*	Increased specific growth rate and survivability	Dey et al. 2017

Interference in quorum sensing

Quorum sensing is a bacterial communication system that leads to the regulation of gene expression in response to the density of signaling molecule (De Almeida et al. 2016). Probiotic microorganisms may inhibit pathogenic infections and reduce biofilm formation through the process of quorum quenching (Defoirdt et al. 2004). Novita et al. (2015) applied probiotic isolates *Lysinibacillus sphaericus*, *Bacillus cereus* and *Bacillus amyloliquefaciens* to disrupt the quorum sensing molecule (N-acylhomoserine lactones) of pathogenic *A. hydrophila* and drastically reduced chronic motile *Aeromonas* septicaemia infection in *C. gariepinus*.

Selection of probiotic

A probiotic strain must pass through certain *in vitro*, *in vivo* and *in silico* analysis before commercialization. The growth pattern, colonizing ability, susceptibility, bio-safety, mode of action and specificity are some most essential determinants. A pilot-scale study can be performed to assess the survivability, efficiency and dose-optimization of candidate probiotic in the host environment. Molecular tracking of marker genes can further validate its authenticity. In this regard, a protocol to screen putative probiotic strain for the cultivation of *C. batrachus* can be proposed (Fig. 2).

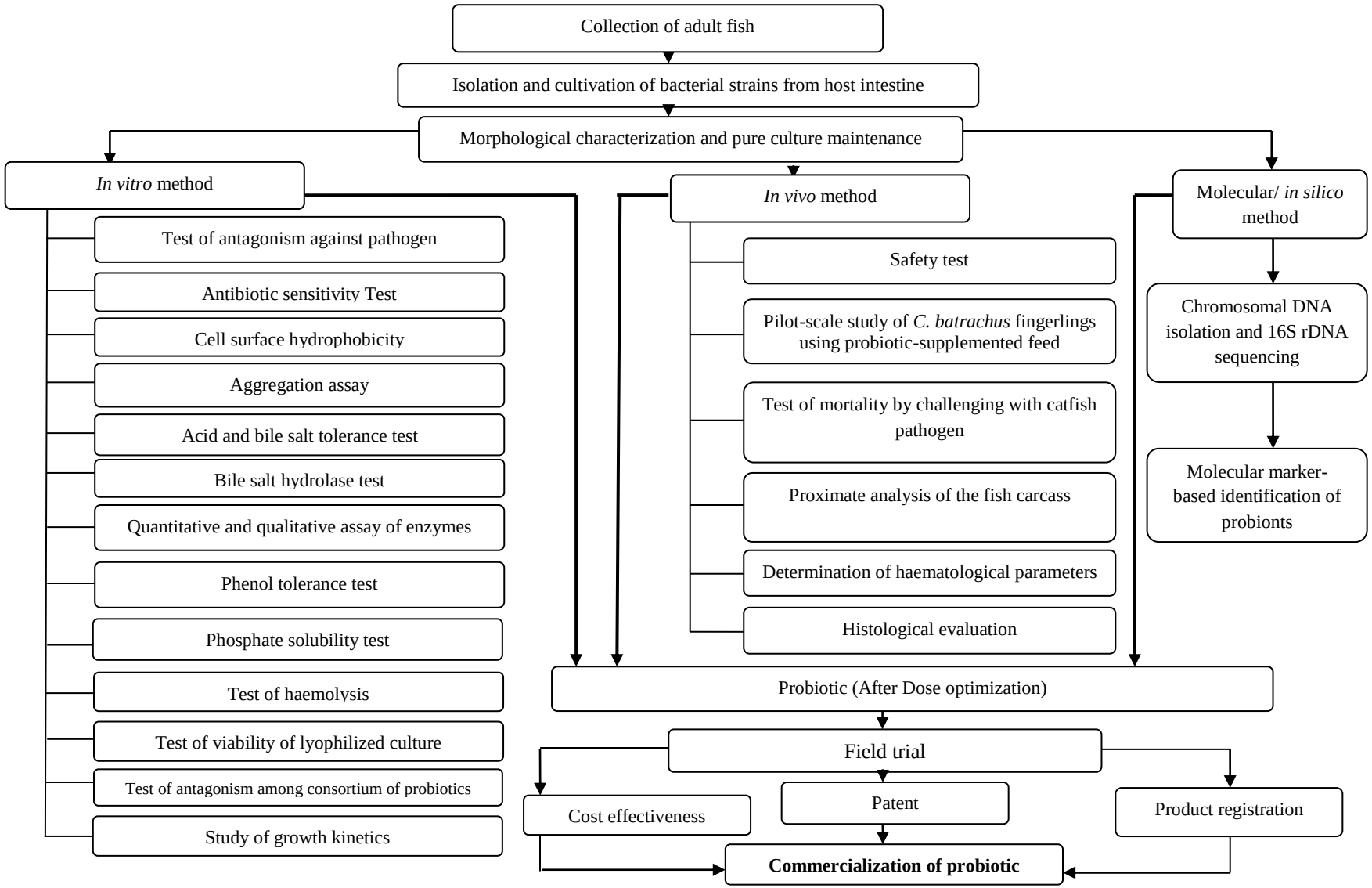

Fig. 2: Diagram for screening of autochthonous probiotic strain for *C. batrachus*.