

A Review on Antimicrobial Activity of Some Bacterial Species in a Fresh Water Fish Pond

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Abstract

Bacteria are constantly being exploited and have been used in farm animals to control the spread of pathogenic microbial species. This is because some specific properties are associated with some of these bacterial species. Such bacterial species are called probiotics and are live harmless microorganisms inhabiting the gut of the host organisms. Various types of probiotics exist and are used for antibacterial, antifungal and antiviral purposes. Mode of action include among others the production of digestive enzymes, competition for nutrients and attachment sites, production of antimicrobial substances and also, their ability to lower the pH of the medium and by regulation of gene expression. Applications of probiotics are done by either introducing the specific bacteria into the digestive system through feed or by adding the beneficial bacteria to the aquarium water. The use of probiotics minimizes the cost expended on antibiotics production, the cumbersome nature of antibiotic production and it enhances the quality of aquaculture. Although some restrictions are observed due to the general suspicion on the use of genetically modified organisms and the ease with which microorganism undergo mutation. These restrictions are further being investigated.

Keywords: Antibacterial; Mutation; Quorum Sensing; Probiotic; Aquaculture; Competition; Substrate

Introduction

Antibiotics are grossly used in aquaculture for the control of various diseases. These antibiotics are synthetic with their associated side effects. It has also functioned in the inhibition of some beneficial microorganisms that assist the metabolic functions of the aquaculture species. The adverse effects of antibiotics culminated in the search for alternative, less expensive and friendlier sources [1].

Bacteria are constantly being exploited and have been used in farm animals to control the spread of pathogenic microbial species. This is because of the intrinsic antagonistic properties associated with some of these bacterial species such as the potential for digestive enzymes' production and competition for substrates, competition for attachment surfaces, production of antimicrobial substances such as toxins, bactericin and also, their ability to lower the pH of the medium due to acid production. Such bacterial species are referred to as probiotics which are living bacteria cells that are used to eliminate or inhibit the growth of bacterial pathogens [2]. The use of probiotics has reduced drastically over reliance on antibiotics in aquaculture industry [3-6]. Hai and Fotedar [7] stated that most bacteria species used as probiotics in aquaculture are isolated from intestine of aquatic organisms. These isolates are non-pathogenic and are mainly members of the lactic acid bacteria.

B. subtilis has been widely used as probiotics (Figure 1) due its non-toxic nature, wide range of nutrients utilization, high degree of enzymes production, and production of antimicrobial compounds [8-11].

The ability of some of these microorganisms to produce spores is added advantage and enables them to withstand adverse environmental condition.

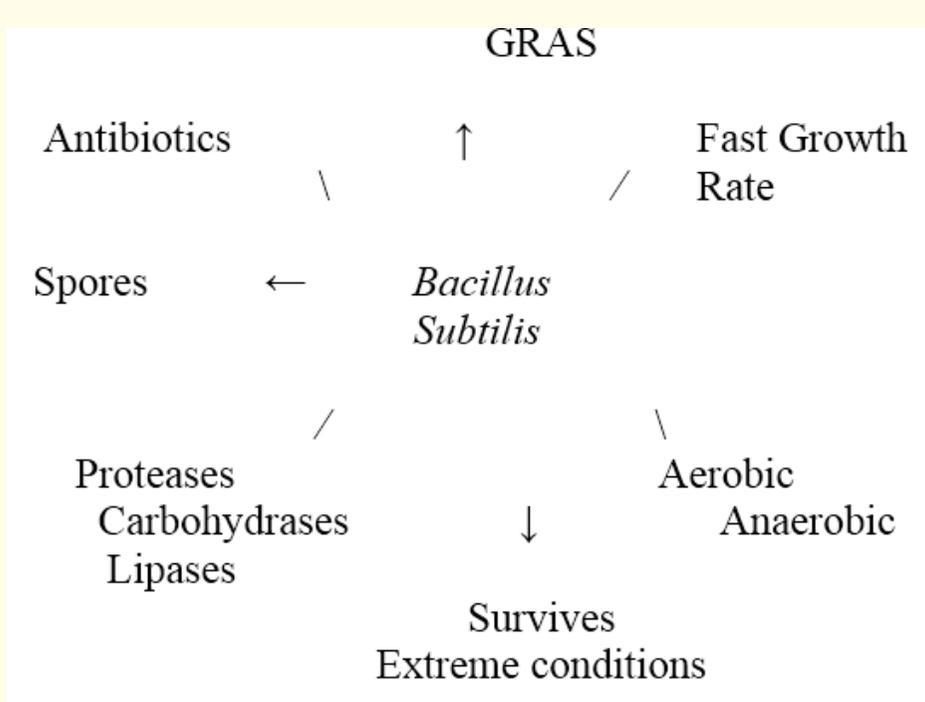


Figure 1: Bacillus subtilis intrinsic properties.

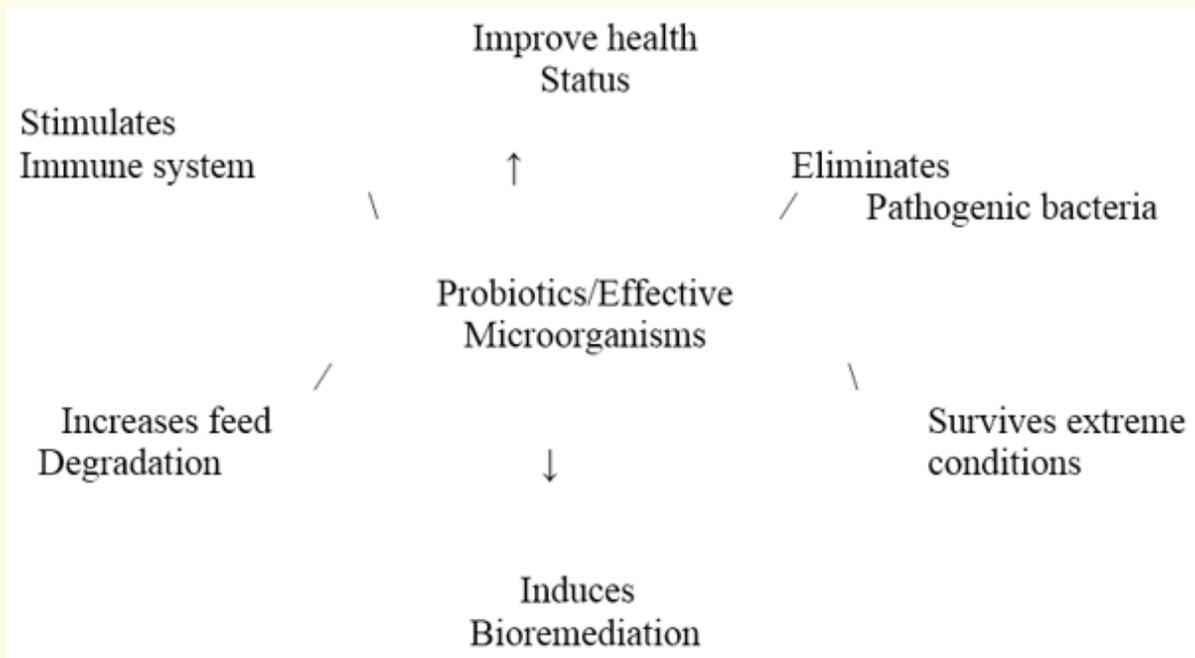


Figure 2: Probiotics/EM capacities in farm animals.

From the above (Figure 2), probiotics/effective microorganisms fit in for living microbial supplement that: (a) promotes host health status, improve immune system; secrete several digestive enzymes and improves quality of environmental parameters that enhances detoxification of waste products.

Fish fed on probiotics grew well without requiring drug treatment [12]. Most probiotics used in aquaculture belong to the genus *Bacillus*, photosynthetic bacteria and yeast, although other genera or species (Table 1) have also been mentioned [13,14]. As stated previously, the lactic acid producing microorganisms have the ability to deliver antagonistic properties against undesirable pathogens by inhibiting

the growth of such pathogens, competing for substrate and generating a non-conductive acid environment (pH= 4 to 4.5).

No.	Probiotic candidates	Aquatic species in which probiotics are used	Beneficial effects	References
Gram-negative bacteria				
1	<i>Aeromonas hydrophila</i>	<i>Oncorhynchus mykiss</i> (Rainbow trout)	<i>Aeromonas salmonicida</i> infection reduced	Irianto and Austi [15,16]
2	<i>Aeromonas media</i> A199	<i>Crassostrea gigas</i> (Pacific oyster)	Reduced <i>Vibrio tubiashii</i> infection	Gibson [17]
3	<i>Pseudomonas aeruginosa</i> , <i>P. synxantha</i>	<i>Penaeus latisulcatus</i> (Western king prawns)	General health and immune status improved	Hai, <i>et al.</i> [7]
Gram-positive bacteria				
4	<i>Arthrobacter</i> XE-7	<i>L. vannamei</i> (Pacific white shrimp)	Alters intestinal microbes	Li, <i>et al.</i> [18]
6	<i>Bacillus megaterium</i>	Shrimp	Immunity improved, intestinal microbes altered and resistant to white spot syndrome virus	Li, <i>et al.</i> [18]
13	<i>Streptococcus faecium</i>	<i>Oreochromis niloticus</i> (Nile tilapia)	As growth promoters	Lara-Flores, <i>et al.</i> [19]
15	<i>Yarrowia lipolytica</i>	<i>Pinctada mazatlanica</i>	Improved growth	Aguilar-Macias, <i>et al.</i> [20]

Table 1: Some species of probiotics used in aquaculture and their beneficial effects.

Besides reducing the environmental pH, they possess the ability to produce other metabolites like enzymes, toxins, carbon dioxide, peroxides or antibiotics, also known as bacteriocins [21] which have antimicrobial activity. Probiotic materials can be used as food supplement in fish culture. Today commercial preparations of useful bacteria like *Bacillus subtilis*, *B. polyriyxa*, *B. negaterium* etc. are available in ready to use packs [13,14] as shown in table 2. Probiotics are generally non-pathogenic.

Brand	Manufacturer	Comments
Naturalle <i>Bacillus subtilis</i>	Wuhan Nature’s Favour Bioengineering Co., Ltd, Wuhan City, China http://www.wuhannature.com	<i>Bacillus subtilis</i> (2 x 10 ¹⁰ CFU/g).
Biozyme	Bio-Pharmachemie Joint-Venture Company, Ho Chi Minh, Vietnam http://www.biopharmachemie.com	<i>Bacillus subtilis</i> and <i>Saccharomyces cerevisiae</i> .
Fubon <i>Bacillus Subtilis</i>	Angel Yeast Co., Ltd. Hubei, China http://www.angelyeast.com	<i>Bacillus subtilis</i> (≥ 20 billion CFU/g).
Bioron	American Pharma International, India http://www.americanpharmainternational.com	Each kg contains: <i>Bacillus subtilis</i> (4.5 x 10 ⁸ CFU), <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>Lactobacillus lactis</i> , <i>L. helveticus</i> , <i>Nitrosomonas sp.</i> <i>Nitrobacter sp.</i> <i>Saccharomyces cerevisiae</i> and <i>Aspergillus oryzae</i> .
Lactomin	American Pharma International, India http://www.americanpharmainternational.com	Each kg contains: <i>Bacillus subtilis</i> (45,000 million CFU), <i>B. licheniformis</i> , <i>Lactobacillus acidophilus</i> , <i>L. sporogenes</i> and <i>Saccharomyces cerevisiae</i> .

Table 2: Probiotic Formula for Aquaculture [10].

Some probiotics activities

Antibacterial activity

Some antibacterial species used in aquaculture are listed in table 3 below each bearing the brand name, manufacturer and its microbial composition.

Antiviral activity

Antiviral activities of some bacteria species have been studied and these studies reveal the use of some gram negative bacteria such as [26] *Pseudomonas*, *Vibrio*, *Aeromonas* spp. and *Coryneforms* against infectious hematopoietic necrosis virus (Kamei, *et al.* 1988). Li, *et al.* [18] demonstrated that feeding shrimps (*Litopenaeus vannamei*) with a *Bacillus megaterium* strain and this boosts resistance to white spot syndrome virus (Balcazar 2003). Harikrishnan, *et al.* [27] studied the use of mixed culture of *Lactobacillus* and *Sorolac* and found that the mixed culture confers resistance against lymphocystis viral disease in Olive Flounder.

Antifungal activity

There are few studies regarding the antifungal effect of probiotics especially the work of Lategan, *et al.* [28] who isolated *Aeromonas* from eel cultured water which exhibited high inhibitory effect on *Saprolegnia* sp. Also, Atira, *et al.* [29] studied *Lactobacillus. plantarum* and found it has inhibitory on *Saprolegnia parasitica* in catfish.

Mode of action probiotic

Competition for attachment site

Attachment sites are prerequisites for pathogenesis of disease causing microorganisms and competition for these sites reduces the chance of survival of most pathogens [30]. According to Adams [31], competition for attachment site is crucial for the survival of microorganisms. This is because, bacteria need adhesion site to be able to colonize the host. Non-pathogenic intestinal microbes compete with the pathogens for adhesion sites making it very difficult for colonization to take place [32].

As such, addition of microbes that will inhibit growth of these pathogens must be done at an early stage of culture to provide better opportunity for competitive exclusion [15]. Attachment of probiotics (Figure 2) may be specific or nonspecific depending on the nature of the attachment sites and the physicochemical agents respectively [33,34].

Production of inhibitory substances

The antibacterial substances produced by most bacteria species inhibit the growth and proliferation of other microbial species hence altering their populations [35]. Toxins and bacteriocins produced by some bacteria species are known inhibitors of some pathogenic bacteria [36]. He added that organic acids and volatile fatty acids produced by most bacteria reduces the pH of the host gastrointestinal tract inhibiting the proliferation of intestinal pathogens [36]. However, 3-benzopyrrole produced some bacteria is known to inhibit growth in some bacteria and fungi [17,28]. Moosavi-Nasab, *et al.* [37] reported the inhibitory effect of lactic acid bacteria on *Listeria* spp when fed on Spanish mackerel. Onianwah, *et al.* [1], the potentials of microorganisms in production of inhibitory substances are responsible for their exploitation at commercial production of these substances.

Competition for nutrients

Microorganisms requires nutrient for survival. These nutrients provide the needed carbon, nitrogen and other forms of energy sources. Availability of nutrients enhances the growth and proliferation of those microbes that can easily access the nutrients. Limitation of nutrients brings in competition. Verschuere, *et al.* [3] and Brown [32] stated that many microorganisms, consume the same nutrients, as such, early colonization by probiotic bacteria reduces the incidence of bacteria pathogens in fish gut. Bacteria, such as *Pseudomonas* and Siderophore synthesizing bacteria have been quite beneficial in this regard [36,38,39].

Regulation of gene expression

Quorum sensing (QS) is a method used by some bacteria species to communicate each other. It is a gene regulation mechanism used by some microbes to control the dynamics of cell population in response to the associated biochemical activities of the cells. The inhibition of this mechanism interferes with infection process in aquaculture [40,41]. Substances such Halogenated furanones, investigated by Manefield, *et al.* [42] has the potential of inhibiting acylated homoserine lactone (AHL) mediated gene expression [36,41,43]. It has been established the some signal molecules from pathogenic bacteria can be broken down by some gram positive bacteria [32,44].

Application of probiotics

Probiotics can be added as viable micro-organism to aquaculture feeds [45]. It can also be through the use of carriers or by inoculating the rearing water with living harmless bacteria species [46]. It can also be through live feed/inert diets which are naturally occurring compounds to facilitate selective stimulation of beneficial gastrointestinal microorganisms. The selection of bacteria species will depend on the fish type, breeding conditions and expected result of the treatment [47]. Factors such as high viability during processing, storage and after gastro-intestinal treatment [45] can enhance quality application of the beneficial microorganisms. However, the stage of treatment is crucial in fish breeding [48]. Appropriate dosage level should also be given consideration and it depends on the probiotic species, host fish species, host physiological status, rearing conditions and the specific goal of feeding application that is, is it for maintaining good health statue, disease resistance or nutrition enhancement [47].

Advantages of Probiotics in Aquaculture

1. They have rapid growth rate and can easily be managed
2. Probiotic microorganisms produces antibacterial substances
3. assist food digestion by production of several digestive enzymes
4. Eliminates the use of antibiotics and hence prevent the possibility of developing drug resistant strains of bacteria.
5. It serves as food supplement to the host by providing some essential nutrients such as minerals and vitamins
6. The use probiotics reduces the cost of running an aquaculture business
7. It is very easy to use and the technology is not cumbersome

Problems associated with the application of beneficial microorganisms

Effective microorganisms and probiotics are generally considered safe and well tolerated [49]. Despite this fact, some constraints are associated with the use of probiotics:

- First is the potential of non-pathogenic microbes transforming into pathogens through mutation thereby causing infection of the host in aquaculture system [50].
- Secondly is the fear that long exposure of pathogens to probiotics may confer on them resistance to some antibiotics thereby making treatment of aquaculture infections difficult [51].
- Thirdly, the non-acceptance of the use of GMOs in aquaculture is a major setback and requires regulation and policy statements reassuring consumers of the safety of probiotics in line with the recommendation of FAO that probiotics are generally regarded as safe [2,11,52].
- Fourthly, there is the issue of overproduction of some antimicrobial agents which can impart negatively on the gut and environmental microbiota culminating in the engineering of new endangered species of microorganisms [11,52-54].
- Fifthly is the fear of harmless commensals of the gut migrating to other unfamiliar parts of the host to cause infection [50].
- Lastly is the fact that despite the inherent fear on the use of GMOs, treatment of some microbial infections (e.g. white spot syndrome virus) with non-genetically modified microorganisms has not been successful. Enlightenment is ongoing aimed at creating awareness on the safety of GMOs as probiotics [55-60].

Summary and Conclusion

Bacteria are constantly being exploited and have been used in farm animals to control the spread of pathogenic microbial species. This is because some specific properties are associated with some of these bacterial species. Such bacterial species are called probiotics are live harmless microorganisms inhabiting the gut of the host organisms. Various types of probiotics exist and are used for antibacterial, antifungal and antiviral purposes. Mechanisms of action include among others the production of digestive enzymes and competition for nutrients and attachment surfaces, production of antimicrobial substances and also, their ability to lower the pH of the medium and by regulation of gene expression. Applications of probiotics are done by either introducing the specific bacteria into the digestive system through feed or by adding the beneficial bacteria to the aquarium water.

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