Fish & Shellfish Immunology 27 (2009) 100–104

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Review

The use of customised probiotics in the cultivation of western king prawns (*Penaeus latisulcatus* Kishinouye, 1896)

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ARTICLE INFO

Article history:
Received 16 February 2009
Received in revised form
11 May 2009
Accepted 14 May 2009
Available online 20 May 2009

Keywords:
Probiotics
Pseudomonas spp.
Western king prawns
Immune responses
Immunostimulants

ABSTRACT

This study presents a comprehensive review of probiotics usage in aquaculture with a specific emphasis on our research series on the effectiveness of the customised probiotics, *Pseudomonas synxantha* and *Pseudomonas aeruginosa* on the cultivation of western king prawns, *Penaeus latisulcatus*. These customised probiotics resulted from tests using five inhibition test methods between the bacteria isolated from two commercial probiotic products and *Vibrio* spp. isolated from western king prawns and other aquatic animals. The results proved the suitability and safety of these probiotics in the cultivation of western king prawns as they conclusively met all the essential requirements for appropriate probiotics. These probiotics have shown similar beneficial effects as the common prebiotics, Bio-Mos® and β-1,3-D-glucan on the growth, survival and immune responses of the prawns. The supplementation of probiotics with the formulated feed was more efficacious and more practical than direct application into the rearing media. The prawns exposed to the combined probiotics were healthier than those exposed to the individual probiotics. *P. aeruginosa* was more effective for improving prawn health than *P. synxantha*. The probiotic-fed prawns were not influenced by *Vibrio harveyi* at 10^3 CFU ml^{-1} for 36 h of challenge. In conclusion, these customised probiotics can be used as appropriate probiotics and as a suitable replacement of antibiotics, for disease control in western king prawn aquaculture.

1. Introduction

Western king prawns are widely distributed throughout the Indo-west Pacific region [1], and throughout warm and temperate waters of the Australian coast [2]. They are considered a prospective species for aquaculture since 1987 [3] because of their established markets in Asia and Australia [4] and their ability to tolerate a wide range of environmental conditions such as salinity and temperature [5,6]. Biological characteristics of this species such as spawning and fecundity [6] or behaviour in effect of food dispersion and crowding [7] have been studied. Recent studies on the culture of this species have been undertaken in Australia by Sang and Fotedar [8,9], Prangnell and Fotedar [10,11], Prangnell [12] and Hai et al. [13], Hai et al. [14], Hai and Fotedar [15]. The prawn cultivation has intensified, which has led to serious losses because of the spread of diseases and the deterioration of environmental conditions [16,17]. As a substantial increase in the regular use of chemical additives and veterinary medicines as preventative and curative measures for disease, leads to antimicrobial resistance among pathogenic bacteria [18–20], past research has shown that one of the benefits of probiotics could be to replace these chemicals and antibiotics [21,22] and therefore, assist in the protection of disease-free aquacultured species [23–25]. Non-pathogenic strains of *Vibrio*, *Aeromonas*, *Pseudomonas* and *Alteromonas* that are known to have antagonistic properties to pathogenic strains, can be used as probiotic strains [25,26].

Although probiotics offer a promising alternative to chemicals and antibiotics in marine prawn culture [21,22,27], the selection of probiotics for specific marine aquatic species has to be considered carefully in order to make them species specific. Research needs to be undertaken on the particular culture conditions of the selected species in order to understand the rationale, preparation and hazards of probiotics [28] from inappropriate species/strains of bacteria selection and/or usage of inadequate population densities [29,30]. Another concern expressed by a few researchers [31,32] is horizontal gene exchange from Gram-negative bacteria to other animals including human. For example, resistance plasmids encoding for antibiotic resistance genes were transferred between pathogen and non-pathogenic Gram negative bacteria in sea water [32]. This paper reviews our past research on the usage of *Pseudomonas synxantha* and *Pseudomonas aeruginosa* on the cultivation of western king prawns in the light of published literature.

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doi:10.1016/j.fsi.2009.05.004

2. Customising probiotics [13]

The inhibition test between the probiotic bacteria isolated from commercially available probiotic products and the *Vibrio* spp. isolated from healthy western king prawns and other aquatic animals was used to evaluate potential probiotics for use in the culture of the prawns and to examine the effects of these probiotics on normal and pathogenic microflora. Eight virulent *Vibrio* spp. isolated from other aquatic animals and 15 *Vibrio* spp., the dominant normal microflora (93.75%), isolated from the prawns, were used for the test of inhibition impact of the selected probiotics on both the virulent and normal microflora. *Vibrio* spp. in their active state have an ability to release organic acids [30] and become resistant to antibiotics such as chloramphenicol, furazolidone, oxytetracycline and streptomycin, and more virulent than the past [32].

Some bacterial species produce a wide range of antagonistic compounds that can be valuable as probiotics [25]. Some probiotics are advertised to contain *Clostridium*, *Pseudomonas putida*, *P. aeruginosa*, but they are usually ineffective in reducing pathogenicity in aquatic species [25]. In our study, only two genera *Bacillus* and *Pseudomonas* were isolated and identified from the two commercial probiotics tested. *Bacillus* spp. are used commercially as probiotics in aquaculture, mainly for prawns [25], while *Pseudomonas* acts as a potential probiotic for marine prawns and has caused growth inhibition of a number of pathogens such as *Salmonella*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio fluvialis*, *Photobacterium damselae* (previously *Vibrio damsela*), *Vibrio vulnificus* and *Aeromonas* spp. [33–35]. Our research indicated that *P. synxantha and P. aeruginosa* were more effective in inhibiting to bacteria isolated from *Penaeus latisulcatus* [13].

Of the five methods: bacteriocin-like inhibitory substance (BLIS), modified BLIS, disc-diffusion, well-diffusion and co-culture, employed for selecting probiotics, the modified BLIS method proved to be the most suitable for determining not only sensitivity/resistance but also the degree of sensitivity between the different *Vibrio* strains and probiotics. The modified BLIS method is also simple and practical compared to the other methods. At the same bacterial cell-density of 10^3 CFU ml^{-1}, this method allows a comparison of inhibitory effectiveness for different probiotics tested on the same range of pathogenic bacteria.

Probiotics at 10^3 CFU ml^{-1} were found to be effective in inhibiting bacteria when tested by the BLIS, modified BLIS and co-culture methods. Probiotic concentrations were required at higher levels to inhibit bacteria using the well-diffusion and disc-diffusion methods. If *Bacillus* strains (probiotics) produced antimicrobial compounds that can inhibit bacteria, then the mortality rates of bacteria could increase [32]. Moreover, probiotics must be applied in advance of the pathogen, because the longer incubation period for the probiotics before the addition of the test bacterium produced greater inhibition zones between the probiotics and *Vibrio* spp. in the modified BLIS, well-diffusion and disc-diffusion methods. If the growth of probiotics is rapid in a chosen medium, an inhibition test might only require one day of incubation before the addition of the test bacteria. Based on our studies of the inhibition tests, the recommended protocol for the selection of probiotics for aquatic animals is the modified BLIS method [13].

3. The customised probiotics on the cultivation of the western king prawns

3.1. Application methods

Administration methods for probiotics need to be considered to get the desired results [36]. Probiotics can be either administered directly into the rearing water or supplemented with the formulated feed. Similar to the results of Skjermo & Vadstein [37] and Azad et al. [38], in our study [14], both probiotic application methods of supplementation with the formulated feed and direct application into the rearing media, were effective in the transfer of the probiotics into the prawns, the former being more practical than the latter in reality. However, the survival and SGRs were higher when the probiotics were supplemented with the formulated feed compared to the probiotics applied into the rearing medium. The prawns that received the probiotics supplemented with the formulated feed were healthier than those receiving the probiotics via the rearing medium as indicated by higher THCs observed in the former.

Probiotics can be applied in mono or mixed culture [39]. A two-probiotic combination improved the growth of prawns [40] and increased the growth of catla (*Catla catla*) larvae and mrigal (*Cirrhinus mrigala*) fry [41]. On the contrary, in our study, the two-probiotic combination did not significantly increase the SGRs of the prawns compared to the individual probiotics, but the prawns exposed to the combined probiotics either through the formulated feed or rearing media had lower HC proportion and higher GC and SGC proportions, and lower bacterial load in the haemolymph, hence were healthier than those exposed to the individual probiotics. *P. aeruginosa* was more effective in improving the prawn health than *P. synxantha*.

V. harveyi is regarded as an opportunistic pathogen [42]. Application of probiotics in advance can prevent the amplification and detrimental effects of *V. harveyi* on the host as the opportunistic pathogen *V. harveyi* has less power under these conditions. Probiotics should be administered at an early stage in the life of the host [37]. *V. harveyi* from broodstock, water, *Artemia* or even bacterial biofilms on the surface of plastic or cement tanks [43,44] may lead to a disease outbreak under stressful conditions [45]. Probiotics application is the main factor in manipulating the indigenous microbiota communities [29], in which a small impact on the growth and mortality of one species may lead to change of species dominance in the communities [46]. The customised probiotics showed a high inhibition test to *V. harveyi* [13]. The probiotic-fed prawns were healthier than those fed without the probiotics [14]. In our study [47], the probiotic-fed prawns survived longer than those not fed probiotics when challenged with *V. harveyi*. Moreover, the probiotics have reduced the effects of *V. harveyi*, allowing the prawns to survive up to 60, 108 and 156 h of challenge with *V. harveyi* at 10^7, 10^5 and 10^3, respectively [47]. The probiotic-fed prawns died if the challenge with *V. harveyi* at 10^3 CFU ml^{-1} lasted longer than 36 h [47]. Therefore, the *V. harveyi* influenced the prawn survival as an opportunistic bacterium in other penaeid prawns.

Overdosage or prolonged administration of probiotics can induce immunosuppression of continuous responses in fish [36], but the effects of overdosage in prawns are yet be conformed. Supplementing a combination of two probiotics (10^5 CFU ml^{-1}) at 20 ml kg^{-1}, Bio-Mos® at 0.5% and β-1,3-D-glucan at 0.2% with the formulated feed improved the SGR, survival, FCR and immune response of the prawns [15]. The probiotic helped the prawns to digest the feed effectively. The prawns consumed less feed leading to significantly lower FCRs when the prawns were fed the probiotics. The SGRs of the prawns in our studies were higher than those reported by Prangnell and Fotedar [10]. Similar to the studies conducted by several authors [48–53], in our study [15], the probiotics increased the SGR, survival and surface structure of the prawn intestines and decreased the FCR of the prawns compared to those without the probiotics. Changing intestinal morphology of smaller crypts with a larger number leads to an increase in the surface structure of the prawn intestines. The nutrient absorption was better when the prawns were fed probiotics leading to a lower FCR than those fed without the probiotics [15].