Use of Microorganisms in Enhancing Shrimp Health and Production

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Introduction

Shrimp production in Malaysia generated approximately RM288 million in 1998, contributing to about 44% of the total aquaculture production (Department of Fisheries 2001). However, the increase in production has not been proportionate to pond acreage mainly because of the occurrence of diseases, water quality deterioration and unsustainable pond management practices. Recent studies on the use of microorganisms in improving water quality, and the control of microbial infections in shrimps have shown promising results (Gil 1995, Rengpipat 1998). However, commercial microbial products showed insignificant increase in shrimp production in the country, probably due to unsuitable environmental conditions for the proliferation of exotic microorganisms (Shariff *et al.* 2001; Devaraja *et al.* 2001). Rengpipat (1998) recommended the use of local isolates for bio-safety reasons as well as to avoid sudden changes in the microbial flora of the ecosystem. The present study focused on the use of indigenous microorganism to improve water quality in shrimp culture ponds and to enhance shrimp health.

Materials and Methods

Indigenous marine bacteria were isolated from sediment and water samples collected from various sites along west coast of Peninsular Malaysia. Isolation of bacteria was carried out according to the method of Austin (1988). Out of 285 colonies initially isolated, three best isolates were selected based on criteria such as secretion of extracellular enzymes and growth at wide range of conditions (temperature, pH, salinity) *in vitro* for the present experiment. The pure colonies of the indigenous isolated marine bacteria were kept in TSA slants (with 1.5% NaCl added) at room temperature for further use. Apart from bacteria, a harpacticoid *Nitocra affinis* was isolated from the seawater samples collected from the coastal waters of Port Dickson. To ensure a monospecies culture, only one beaker with the gravid female were grown continuously for several generations. The copepod was then mass produced in plastic basins and tested on *Peneaus monodon* as a potential live feed substitute.

Results and Discussion

Effect of combination of two bacteria showed that rate of ammonia reduction was higher compared to individual species under simulated shrimp pond conditions. *Bacillus* spp. were found to contain fatty acids mainly of saturated and monoenoic group except for one isolate, which had fatty acid of polyenoic group. The *Bacillus* spp. were found to secrete protease, gelatinase, amylase and lipase extracellular enzymes. All the *Bacillus* isolates were sensitive to most of the antibiotics tested. These isolates were compatible with each other in mixed culture conditions. Shrimp fed with bacterial additives showed the following positive indices; better growth (weight gain, 29.9 mg vs 25.4 mg; length, 21.9 mm vs 21.2 mm; specific growth rate 6.1% vs 4.4%), and survival (55.3% vs 31.3%) compared to the control fed with commercial shrimp feed. In addition, shrimp fed on bacterial supplemented diet had higher survival rate (15%) when challenged with the white spot virus (WSSV) whereas shrimp fed on artificial diet showed 100% mortality. *Bacillus* spp. had the ability to suppress the growth of pathogenic bacteria isolated from shrimp and *Artemia* cyst. They inhibited as well as excluded all the pathogenic vibrios tested by disc diffusion, streak plate and common broth methods. The isolates were tested for their non-pathogenecity to shrimp postlarvae. The selected *Bacillus* isolates satisfied the criteria to qualify them for both as a bioremediator as well as a bacterial feed supplement. Further studies on other suitable aquatic microorganisms to complement the effectiveness of *Bacillus* spp. are needed to develop effective bioremediation products. Apart from that, *Nitocra affinis* fed *P. monodon* showed highest survival rate (60.6%) compared to *Artemia* and commercial feed. Likewise, highest specific growth rate was achieved by shrimp fed with *N. affinis*. On the other hand, the fatty acids of *N. affinis* was dominated by polyunsaturated fatty acids (PUFA) (22:6n-3) forming 19.5% of the 27 total fatty acids compared to *Artemia* and commercial feed. In fact, *N. affinis* contained the highest amount of PUFA and highest n-3/n-6 ratio amongst the three diets. Analysis of the copepod fed shrimp showed significantly higher amount of long chain PUFA, both of the n-3 and n-6 series fatty acids compared to artificial diet fed larvae. Thus *N. affinis* has a potential to be used as a substitute live feed for *P. monodon* due to their broad size range to cater for post larval stages and high PUFA contents.

Conclusions

The study illustrated that the isolated *Bacillus* spp. had the ability to lower ammonia in shrimp culture system. Moreover *Bacillus* spp. incorporated diet fed to *P. monodon* had better growth and survival rate compared to the control when challenged with white spot syndrome virus. The isolated *Bacillus* spp. also had the ability to suppress growth of pathogenic bacteria isolated from *Artemia* sp. Moreover, the harpacticoid *N. affinis* has a potential to be used as a substitute live feed for *P. monodon*. Further studies are needed to source other microorganisms as compatible partners to *Bacillus* spp. to fine tune the bioremediation as well as the probiotic product

Benefits from the study

Trials pertaining to lowering of ammonia in shrimp culture system and growth and survival of postlarvae have shown encouraging results. Hence, there is a possibility to develop two products, bioremediation as well as feed additive, using the isolated microorganism. This will help in producing healthy and disease free shrimp and minimize pollution in coastal waters leading to a sustainable and healthy shrimp culture in Malaysia. Moreover it will help in reducing the import of harmful non-indigenous bacterial products from overseas, thus saving the country foreign exchange. General benefits of the study include:
Novel method of purification of microalgae
Improves water quality and enhances aquaculture production
Prevents pollution of public waters
Cheaper source of essential amino acids and polyunsaturated fatty acids
Reduce dependence on imported microalgae and earn foreign exchange
Potential for commercialization
A total of 17 papers were published, 3 in international journals and 14 in proceedings. Three more manuscripts have been submitted.

Patent(s), if applicable :

Nil

Stage of Commercialization, if applicable

A new medium for microalgal growth was developed from aquaculture sediment. The rich extract can be used to mass culture pure and high quality microalgae for feed additives, pharmaceutical and cosmetic products. The advantages of the extract are:
Cost effective being cheaper than standard medium
Pure algal growth in the extract is significantly higher ($p>0.05$) than the standard medium
Microlgae contains higher levels of polyunsaturated fatty acids as compared to those grown in standard media
Further experiments are being conducted to fine tune the bioremediation as well as the shrimp feed additive. As such, funds from the National Biotechnology Directorate (NBD) have been obtained to continue the work keeping in view to commercialize the products.

Project Publications in Refereed Journals

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Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Devraja, T.N.	Investigation on Indigenous *Bacillus* Isolates with Bioremediation Properties for Improving Water Quality and Shrimp Health in Malaysian Aquaculture	Bioremediation	Ph.D.	Nov. 2002
Hamid Rezai Marnani	Ecological Studies on Zooplankton from the Straits of Malacca with Special Reference to Copepods	Aquatic ecology	Ph.D.	March 2003

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