Probiotics and sea cucumber farming

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Abstract

With the development of sea cucumber farming, new diseases are expected to appear in the near future. To prevent aquaculture-related diseases, antibiotics are generally used in large quantities although their unlimited application results in the appearance of new virulent pathogens. Probiotic technology is rapidly becoming popular due to its effectiveness against pathogenic microorganisms. This work emphasises the use of probiotics against pathogens in the sea cucumber farming industry.

Introduction

Sea cucumbers are the most commonly consumed echinoderms and have been eaten since ancient times. Although holothurian fishing has a long history, sea cucumber aquaculture has only developed in recent decades. It is now well established in many countries such as Australia, China, the Galápagos Islands, Indonesia, Japan, Malaysia, and the Philippines (see Conand 2004 for review). The raw products were consumed within the producing countries, while processed products were exported mainly to China, Hong Kong and Taiwan (Conand 2004; Vannuccini 2004).

In addition to their good flavour, sea cucumbers are also eaten for their medicinal value. They are commonly used for treating weakness, impotence, constipation and frequent urination (Hamel and Mercier 2004). They are also remarkably rich in vitamins, trace elements, and polysaccharides (condroitin sulfate), which reduce arthritis pain and inhibit viral activities, and saponin glycosides that inhibit cancer activities (Hamel and Mercier 2004).

The rapid increase in market demand has resulted in the overexploitation of natural sea cucumber populations all over the world. As a result, sustainable industries with modern hatchery techniques have been established in several countries. Because of the low oxygen consumption of sea cucumbers, some industries have accomplished financially profitable polyculture farming (shrimp and sea cucumber) without extra aeration or frequent water exchange (Hamel and Mercier 2004). The quick spread of intensified farming has led to an increase of various pathogenic diseases, which have become a major limiting factor in the industry. A lack of information regarding diseases and preventive measures makes it difficult to ascertain the expected profit of the industry if faced with a disease outbreak.

Holothurians are easily infected by parasites, including protozoans, bacteria and metazoans (Becker et al. 2003; Eeckhaut et al. 2004). The high density of animals in hatchery tanks and ponds facilitates the spread of pathogens, and the aquatic environment — with regular applications of protein-rich feed — is ideal for culturing pathogenic and non-pathogenic microorganisms. Even when pathogenic bacteria or viruses are not present, farmers use antibiotics as prophylactics in large quantities. This has led to an increase in bacteria that are resistant to multiple antibiotics and to an increase in more virulent pathogens. Therefore, the use of beneficial bacteria that would reduce pathogenic bacterial development by a competitive process is a better solution than antibiotic applications. This work discusses the possibility of using probiotics as an "eco-friendly", biological method of disease control in sea cucumber farming.

Diseases and associated microorganisms of sea cucumbers

Since sea cucumber farming is a relatively new industry, the emergence of disease outbreaks is not well understood in contrast to diseases observed in other economically important aquaculture industries. However, the ''Advances in sea cucumber aquaculture and management'' workshop, held in China in 2003, pointed out several diseases that appear worldwide and within different species.

Very few studies have been carried out to identify the causal agents of sea cucumber diseases. Zhan and Yu (1993) identified parasitic sporozoans. Most of these parasites were seen in the hemal system and in the gut of sea cucumbers.

To identify microbial diseases, Sun and Chen (1989) carried out studies on Ling Shan Island (China) and isolated 11 genera of bacteria, from the foregut, hindgut, coelomic fluid and the integument of *Stichopus japonicus*. The bacteria belonged to the following genera: *Vibrio*, *Pseudomonas*, *Neisseria*, *Acinetobacter*, *Flavobacterium*, *Arthrobacter*, *Micrococuus*, *Xanthomonas*, *Corynebactterium*, *Caulobacter*, and *Alcaligenes*. Four genera of yeast — *Torulop-*

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sis, Rhotorula, Cryptococcus and *Debaryomyces* — were also found in sea cucumbers and *Achromobacter* and *Bacillus* were the dominant genera in muddy environments (Sun and Chen 1989). *Bacillus* strains are commonly found in mud but a few of them are found in *Stichopus japonicus* species (Sun and Chen 1989).

At the aqualab hatchery in Madagascar, a highly contagious skin ulceration disease appeared on juvenile *Holothuria scabra* and spread quickly (Becker et al 2003). This disease has also been reported in *Aspostichopus jappononicus* in China, in *Isostichopus fuscus* in Ecuador, and in *H. scabra* in Australia and New Caledonia (Becker et al. 2003). The results of this study indicate that a combination of events and agents, including bacteria, are required to induce the disease. *Vibrio* species (close to *V. harvey* and *V. alginolyticus*), bacteroid species and "alpha" proteobacteria were identified from the diseased animals.

Due to the availability of sea cucumbers in China, studies are frequently carried out to improve management and to control diseases. Wang et al. (2004) illustrated several non-reported diseases of *Apostichopus japonicus*, including syndromes of rotting edges, stomach ulcerations in auricularia stages, and autolysis of young juveniles that were caused by bacterial agents. Skin ulcerations, epidermal erosion, and body oedema were triggered by various pathogens including bacteria, fungi and parasites during outdoor cultivation. The authors noted that in 2002–2004 the high mortality rate caused great economic loss, which was due to three epidemic diseases called "the syndrome of rotting edges", "the syndrome of off-plate" and "the syndrome of skin erosion". All were caused by *Vibrio* species and the results concluded that vibriosis is the main disease in farmed sea cucumbers. The authors recommended the use of antibiotics as preventive measures. *Vibrio* grow attached to algae and many reach high population densities after being ingested with algae and then expulsed from digestive tubes with lysed algae in faecal pellets; they are gut bacteria in aquatic animals, including zooplanktons (Sun and Chen 1989).

Efficacy of probiotics in disease management

A probiotic can be defined as a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal (microbial) balance (Fuller et al 1989). Verschuere et al. (2000) expanded the definition to include a live microbial adjunct that has a beneficial effect on the host by modifying its associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, and by improving the quality of its ambient environment.

With successful evidence from past decades, Irianto and Austin (2002) revealed that microalgae (tetraselmis), yeasts (*Debaryomyces, Phaffa* and *Saccharomyces*), gram-positive bacteria (*Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Micrococcus, Streptococcus* and *Weissella*) and gram-negative bacteria (*Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas* and *Vibrio*) and immuno-stimulants (polysaccharides, glycans) can be used as successful probiotics. Unfortunately, no research has been conducted to investigate the application of probiotics to sea cucumber farming.

The humic substances found in bottom sediments, sea and lake waters, soils, peat and coals, resulting from the decomposition of organic matter, particularly dead plants, consist of a mixture of complex macromolecules with polymeric phenolic structures (Flaig et al. 1975; Kupryszewski et al. 2001). The antimicrobial characteristics of 12 preparations of humic substances isolated from seawater, marine bottom sediments and lake water were examined by Kupryszewski et al. (2001). The focused humic substances, humic acids and fulvic acids exhibited varied anti-microbial activities that can be used successfully in disease control. Verschuere et al. (2000) reported that the use of antibiotics does not allow microbial control and may result in an unfavourable change in microbiota. The establishment of a normal gut microbiota may be regarded as complementary to the establishment of the digestive system and under normal conditions serves as a barrier against invading pathogens.

Recently, Gorski et al. (2003) observed that bacteriophage bind to animal cells, including lymphocytes, and perhaps exert immuno-modulatory actions. It is reported that bacteriophage has a dramatic effect on infection, resulting in a 98% reduction in bacterial titre. These bacteriophages could be used as a simple vaccination strategy involving oral delivery of phage tablets or liquid suspensions (Gorski et al. 2003). Verschuere et al. (2000) reported that most probiotics proposed as bio-control measures are lactic acid bacteria (e.g. *Lactobacillus, Carnobacterium*), V*ibrio* (e.g. *V. alginolyticus*), *Bacillus* and *Pseudomonas.* A variety of *Vibrio* phages are prevalent in the Gulf of Mexico (Kellogg et al 1995). Moebus and Nattkemper (1983) revealed that 362–366 phage-sensitive bacteria isolated from the Atlantic belonged to the family Vibrionaceae and that 280 of them were V*ibrio* species.

The activities of microorganisms as probiotics in aquaculture or as biological control agents were described by Verschuere et al. (2000) and Irianto and Austin (2002). In particular, the use of probiotics in shrimp farming is highly effective and used worldwide. Ackermann (2003) illustrated that most pathogenic bacteria can be controlled by using their host phage (Table 1).

Table 1. Host range of major phage group (Ackermann 2003)

Phage group	Bacterial group or genus
Caudovirales	*Eubacteria, Euryarchaeota*
Microviridae	*Enterobacteria, Bdellovibrio, Chamydia, Spiroplasma*
Coticoviridae	*Alteromonas*
Tectiviridae	*(a) Enterics, Acinetobacter, Pseudomonas, Thermus, Vibrio* *(b) Bacillus, Alicyclobacillus*
Leviviridae	*Enterics, Acinetobacter, Caulobacter, Pseudomonas,*
Cystoviridae	*Pseudomonas*
Inoviridae	
(a) Inovirus	*Enterics, Pseudomonas, Thermus, Vibrio, Xanthomonas*
(b) Plectrovirus	*Acholeplasma*
Plasmaviridae	*Acholeplasma*
Lipothrixviridae	*Crenarchaeota: Acidians, Sulfolobus, Thermoproteus*
Rudiviridae	*Crenarchaeota: Sulfolobus*
Fuselloviridae	*(a) Crenarchaeota: Aciiaians, Sulfolobus* *(b) Euryarchaeota: Methanococcus, Pyrococcus*

Tovar et al. (2002) pointed out that some strains of yeast produce polyamines, which enable the yeast to adhere to intestinal mucus. In this study it was found that the yeast *Debaryomyces hansenii* HF1 (DH), isolated from fish guts, has the ability to secrete polyamines in significant amounts.

Villasin and Pomory (2000) developed a method to extract substances with antibacterial activities from sea cucumbers. A methanol acetone extract from the body wall of the sea cucumber *P. parvimensis* had antibacterial properties against two species of bacteria (*Bacillus subtilis* and *Enterococci coli*). Ridzwan et al. (1995), tested extracts from the sea cucumber *H. atra, H. scabra* and *Bohadshia argus* against seven species of bacteria and found that lipid and methanol extracts had no inhibitory activity, though a phosphate buffered saline extract did have inhibitory activity. Because sea cucumbers lack a well-developed immune system and can ingest pathogenic bacteria together with food, some form of active antibacterial substances must be present in the body for defence (Ridzwan et al. 1995).

Discussion

Because oceans cover three-quarters of the planet, marine phages are probably the most prevalent life forms. Approximately 2100 bacteriophages were listed in 1981, and this amount increases annually by about 100 for a present total of more than 4000. Several bacteriophages have been intensively investigated at the molecular level and the interaction between these bacteriophages and their hosts under laboratory conditions is well understood (Frank and Russell 2000). The majority of pathogenic bacteria associated with sea cucumbers are common pathogenic species and possible bacteriophages have been investigated. Further research should concentrate on controlling sea cucumber diseases by using probiotics. The use of disinfectants and antimicrobial drugs has had limited success in the prevention or cure of bacterial diseases because of their ability to produce resistant genes and transfer genes from generation to generation. However, production and use of probiotics in successful disease control depends on an awareness of the relationship between particular species and strains of bacteria.

Management of water quality in an appropriate manner enhances the health of cultured animals. Disinfection methods normally used in wastewater treatment on land-based fish farms are ultraviolet radiation, ozonation and chlorination. Because effluent from fish farms normally consists of suspended solids, organic matter and different strains of harmful microorganisms, it is essential to focus on disinfection of effluent and pond environments to ensure they are free of pathogenic microorganisms, thus avoiding frequent disease outbreaks. Bomo et al. (2003) reported on the use of low-cost infiltration systems, such as sand filters, as an alternative to environmentally hazardous chemicals for the disinfection of fish-farm wastewater.

Some associated microorganisms present in sea cucumbers exhibited the ability to secrete chitinase (Sun and Chen 1989) and some other polysaccharides. Chitin is a natural polymer that exhibits antibacterial activities, and more attention should be paid to using natural substances as disease control agents. Many other extracts of sea cucumbers indicate antimicrobial activities that can be used in disease control methods.

There is evidence that diseases occurring in this new industry can be controlled in an eco-friendly manner to avoid outbreaks and develop antibiotic-resistant pathogens in the future. The authors of this review have isolated several marine bacteria and yeast species associated with sea cucumbers; two strains of yeasts were taxonomically identified as *Yarrowia lipolytica* and *Candida tropicalis*, which are capable of producing phytase. Identified yeast strains were deposited at the National Center for Biotechnological Information (NCBI), USA, under the accession numbers DQ 438177 and DQ 515959, respectively. The authors are conducting further research on these microorganisms.

References

Ackermann H.W. 2003. Bacteriophage observations and evolution. Research in Microbiology 154:245–251.

Becker P., Gillan D., Lanterbecq D., Jangoux M., Rasolofonirina R., Rakotovao J., Eeckhaut I. 2003. The skin ulceration disease in cultivated juveniles of *Holothuria scabra* (Holothuroidea, Echinodermata). Aquaculture 242:13–20.

Bomo A.M., Husby A., Stevik T.K. and Hanssen F.J. 2003. Removal of fish pathologenic bacteria in biological sand filters. Water Research 37:2618–2626.

Conand C. 2004. Present status of world sea cucumber resources and utilization: An international overview. p. 13–25. In: Lovatelli A., Conand C. Purcell S., Uthicke S., Hamel J.-F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Reports No. 463, FAO, Rome. 425 p.

Eeckhaut I., Parmentier E., Becker P., Gomez da Silva S. and Jangoux M. 2004. Parasites and biotic diseases in field and cultivated sea cucumbers. p. 311–325. In: Lovatelli A., Conand C. Purcell S., Uthicke S., Hamel J.-F. and Mercier A.(eds). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Report No.463, FAO, Rome. 425 p.

Flaig W., Beutelspracher H. and Rietz E. 1975. Chemical composition and physical properties of humic substances. In: Gieseking J.E. (ed). Soil components. New York: Springer. 1211 p.

Frank T.R. and Russell T.H. 2000. Bacterial viruses and hosts: Influence of culturable state. In: Colwell R.R. and Grimes D.J. (eds). Non-culturable microorganisms in the environment. Washington. D.C: ASM press,.

Fuller R. 1989. A review: Probiotics in man and animals. Journal of Applied Bacteriology 66: 365–378.

Gorski A., Nowaczyk M., Weber-Dabrowska B., Kniotek M., Boratynski J., Ahmed A., Dabrowska K., Wierzbicki P., Switala-Jelen K. and Opolski A. 2003. New insights into the possible role of bacteriophages in transplantation. Transplantation Proceedings 35:2372–2373.

Hamel J.F. and Mercier A. 2004. Synchronous gamete maturation and reliable spawning induction method in holothurians. p. 359–372. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.-F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Reports No. 463, FAO, Rome. 425 p.

Irianto A. and Austin. B. 2002. Probiotics in aquaculture. Journal of Fish Diseases 25:1–10.

Kellogg C.A., Rose J.B., Jiang S.C., Thurmond J.M. and Paul J.H. 1995. Genetic diversity of related vibriophages isolated from marine environments around Florida and Hawaii, USA. Marine Ecology Progress Series 120:89–98.

Kupryszewski G., Pempkowiak J. and Kedzia A. 2001. The effect of humic substances isolated from a variety of marine and lacustrine environments on different microorganisms. Oceanologia 43(2):257–261.

Moebus K. and Nattkemper H.1983. Taxonomic investigations of bacteriophage sensitive bacteria isolated from marine waters. Helgoland. Meeresunters 36:357–373.

Ridzwan B.H., Kaswandi M.A., Azman Y. and Fuad M. 1995. Screening for antimicrobial agents in three species of sea cucumbers from coastal areas of Sabah. General Pharmacology 26(7):1539–1543.

Sun Y. and Chen D. 1989. Study of in vitro and in vivo physical characters of different types of microbes in sea cucumber. Oceanologia et Limnologia Sinica 20:300–307. (in Chinese).

Tovar D., Zambonino-Infante J.L., Cahu C., Gatesoupe F.J., Vazquez-Juarez R. and Lesel R. 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass larvae. Aquaculture 204:113–123.

Vannuccini S. 2004. Sea cucumbers: A compendium of fishery statistics. p. 399–414. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.-F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Report No. 463, FAO, Rome. 425 p

Verschuere L., Rombaut G., Sorgeloos P., and Verstraete W. 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews 64:655–671.

Villasin J. and Pomory M.C. 2000. Antibacterial activity of extracts from the body wall of *Parastichopus parvimensis* (Echinodermata: Holothuroidea). Fish and Shellfish Immunology 10:465–467.

Wang Y.G., Zhang C.Y., Rong X.J., Chen J.J., Shi C.Y., Sun H. L. and Yan J.P. 2004. Diseases of cultured sea cucumber (*Apostichopus japonicus)* in China. p. 297–310. In: Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.-F. and Mercier A. (eds). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Report No. 463, FAO, Rome. 425 p

Zhan W.B. and Yu K.K. 1993. Diseases of sea cucumber and sea urchin. Journal of Oceanology and Limnology 1:95–100. (in Chinese).