A microbiological study of a local population of *Saccostrea sp.* found in Tangalle

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Abstract

Owing to the considerable amount of oyster resources reported in the coastal waters of Sri Lanka, oysters can be popularized as an alternative source of animal protein among Sri Lankans and as an industry. However, the microbiological safety needs to be assessed before routine consumption. The aim of this study was to study the bacterial profile of a local population of oyster sp. commonly found in Tangalle in order to assess its microbiological safety. Oysters were collected aseptically, from a rocky shore at Pallikkudawa beach, Tangalle and the species was identified as *Saccostrea cucullata* based on the morphological features. The soft tissues of each oyster was taken out aseptically and was homogenized using 0.09% saline. Each homogenate was cultured on nutrient agar, MacConkey agar and Thiosulphate Citrate Bile Salts (TCBS) agar and was incubated at 37^{0}C overnight. Approximately 5 g of oyster tissue was pre-enriched in peptone water and was then cultured on MacConkey agar. Organ cultures were also made on MacConkey agar. Identification of bacteria was done by studying the colonial morphology, colour changes of the medium, microscopic examination of Gram-stained smears and biochemical tests. *Klebsiella pneumoniae, Proteus* sp., *Vibrio alginolyticus* and *Bacillus* spp. were isolated and identified.

Keywords: Oyster, *Saccostrea cucullata*, bacteria, Vibrio

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Introduction

Oysters are filter-feeding bivalves and the most harvested shellfish in the world (Piyathilaka et al. 2012). Farming oysters for raw or partially-cooked consumption is an emerging and export-oriented industry in Sri Lanka (Indrasena and Wanninayake,1986). Since oysters are filter-feeders, pathogens can be greatly concentrated and microbiological studies are useful in reducing the risk of consuming oysters. The aim of this study was therefore to study the bacterial profile of a local population of oyster sp. commonly found in Tangalle in order to assess its microbiological safety.

Materials and Methods

Oysters were collected aseptically using a sterile knife, from a rocky shore at Pallikkudawa beach, Tangalle. The population was identified as *Saccostrea cucullata*, based on the morphological features. Samples were collected from October to December, 2015, for a period of two months. Oysters were shucked and soft tissues including gill and mantle and intervalvar fluid were transferred aseptically to sterile bags. The tissues were then homogenized in 0.09% saline, using a bag mixer (Inter Science, France).

Each homogenate was cultured on nutrient agar, MacConkey agar and Thiosulphate Citrate Bile Salts (TCBS) agar and was incubated at 37 °C overnight. Approximately 5 g of tissue from each oyster was pre-enriched in peptone water and was then cultured on MacConkey agar. Organ cultures were also made on MacConkey agar. Identification of bacteria was done by studying the colonial morphology, colour changes of the medium, microscopic examination of Gram-stained smears and biochemical tests using Enterobacteriaceae identification test kit (HIMEDIA KB003, India) and Vibrio identification test kit (HIMEDIA KB007, India). Bacterial colonies which were suspected to be Pseudomonas and Salmonella were cultured in TSI agar and was incubated at 40°C 72 hours.

Results and Discussion

The Enteric bacteria demonstrated the following results (table 1) on culture media, Gram-stained smears and with biochemical tests. The large, white, flat colonies with irregular edges which showed chains of Gram-positive rods were identified as *Bacillus* spp. The yellow colonies on TCBS agar were identified as *Vibrio alginolyticus* based on the biochemical tests and demonstration of Gram-negative curved rods. Another type of green colonies were observed on TCBS agar which was also thought to be a Vibrio, however, the species could not be determined by the biochemical tests carried out. Overall, *Klebsiella pneumoniae,Proteus* sp., *Vibrio alginolyticus* and *Bacillus* spp. were isolated and identified in this study. Previous studies have also isolated *V. parahaemolyticus, V. anguillarum, V. harveyi*, and *V. vulnificus* along with other *Vibrio* spp., from *Saccostrea cucullata* (Chen Mei *et al.* 2000). Moreover, *Pseudomonas* spp. and *Bacillus* spp. have been identified in *Crassostre gigas* (Hernandez and Olmos, 2006)

Table 1. Results of culturing and biochemical tests for carried out for Enteric bacteria

MacConkey agar	Gram-stained smears	Indole test	Methyl Red test/Urease test	Species
Pink, mucoid colonies	Gram-negative rods	(-) ve	(-) ve	*Klebsiella pneumoniae*
Pink/colourless colonies on yellow medium	Gram-negative rods	(-) ve	(+) ve	*Proteus spp.*

Conclusion

Klebsiella pneumoniae, Proteussp., *Vibrio alginolyticus* and *Bacillusspp*. were present in the local population of *Saccostrea cucullata*. This indicates the need of taking adequate precautionary measures such as relaying & depuration and high-pressure processing before using farmed oysters for consumption.

References

Chen, Mei 2000. The study of Vibro species in some cultured marine animals in Shandong coastal water and the control of the Vibrio diseases, Shangdong Fisheries 06.

Hernandez, Z. G. and Oimos, S.J. 2006. Identification of bacterial diversity in the oyster Crassostreagigas by fluorescent in situ hybridization and polymerase chain reaction, *Journal of Applied Microbiology***100(4):** 664-72.

Indrasena, W. M. and T. B. Wanninayake 1986. Settlement and growth of oyster spat in Trincomalee Bay, Sri Lanka, p 59–62. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos (eds.): The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Piyathilaka, M.A.P.C., Hettiarachchi,M. and Wanninayake,W.M.T.B. 2012. Growth and health status of cultured edible oyster, *Crassostreamadrasensis* (Preston) in the Panadura estuary, Sri Lanka **40:** 201-210.