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Assessment of bio-accumulation of bacteria in oysters from shellfish growing waters in Ashtamudi Lake (Kerala, India): A RAMSAR wetland

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HIGHLIGHTS

- In India, this is the first-ever assessment, which offer an insight on the accumulation of bacteria in farmed oysters.
- We investigated the dynamic process of microbial bio-accumulation and how it is influenced by rainfall.
- Bio-accumulation was high during the pre-monsoon season when the Lake is brackish.
- Salinity and temperature play a major role in the survival of coliform bacteria in the shellfish growing water.
- We can reduce input costs in the laboratory by analysing faecal coliform as a quality indicator instead of using *E. coli* as an indicator.

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ABSTRACT

Oysters are commercially cultured from the Ashtamudi Lake in India, and support 12,000 tonnes of bivalve fisheries/year. Oysters and oyster growing waters were sampled from July 2012 to June 2013 for analysis of total coliforms (TC), faecal coliforms (FC), *Escherichia coli*, faecal *Streptococci* (FS) and total plate counts (TPC). *E. coli* MPN values in oyster growing waters were below the threshold limits set by the USFDA and EU during the months of December to April. Seasonally, the highest MPN values for *E. coli* were obtained during the monsoon season (June–September), and this trend gradually decreased during the post-monsoon (October–January) and pre-monsoon (February–May) periods. *E. coli* displayed a significant ($p < 0.01$) variation in accumulation during different seasons. A strong negative correlation ($R^2 = -0.70, p < 0.05$) between temperature and *E. coli* numbers in oysters was observed, while rainfall and *E. coli* were positively correlated ($R^2 = 0.695, p < 0.05$). Hence, we strongly recommend depuration and proper cooking of oysters before consumption during the monsoon season.

1. Introduction

Coastal estuaries are highly productive ecosystems that can sustain several human activities. These aquatic environments are among the most extensively used for the commercial production of bivalves (Almeida and Soares, 2012). The microbiological pollution of coastal waters is a major problem, especially in shellfish growing areas where contamination may exist, with sewage discharges including sewage outfall, combined sewer overflows and rainwater discharges the most significant (Lee et al., 2003; Oliveira et al., 2011). As filter feeding organisms, bivalves can concentrate contaminants from the surrounding water including microorganisms that can cause several infectious diseases in humans (Muniain-Mujika et al., 2002; Brands et al., 2005).

Consumption of shellfish containing harmful microorganisms may pose a significant public health risk (Fleming et al., 2006); thus, their hygiene and sanitary control is extremely important and is legislated in many countries (Lees, 2000; Oliveira et al., 2011). Classification of growing areas by sanitary survey and monitoring of *E. coli*/faecal coliforms at an appropriate frequency based on the risk of contamination is prevalent in Europe. These classifications are based on Directives 923/79/CEE, which serve as guidelines to control the levels of microorganisms in both shellfish and the overlying waters (EC, 2004a). These standard guidelines are important for the regulation of shellfish harvesting and public health. In India, such standards have not yet been developed because until recently there was no significant consumption of shellfish. However, shellfish cultivation has substantially increased in recent decades and has become an important industry for rural coastal communities. Indigenous species of clams, mussels and oysters are harvested from natural populations, and the characteristics of these bivalve molluscs make them suitable for

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cultivation. In India, the cultivation of the oyster *Crassostrea madrasensis* is performed mainly in the estuaries and backwaters of Kerala, particularly from Ashtamudi Lake. The production of the oyster *C. madrasensis* was nil in the year 1990 and reached 4700 tonnes in 2013 (FAO, 2013). Recently, the edible oysters are very popular as raw and processed food in the South Indian states, particularly in Kerala and Goa (Jana et al., 2013), where large number of foreigners visiting every year. The trade of live oysters also has a high demand in high-end restaurants, and their market value has increased tremendously from Rs 0.5/oyster to 7.5/depurated oyster (Mohamed and Kripa, 2013). Under these circumstances, the shellfish harvesting areas in India have to be classified as suitable to harvest for direct human consumption based on purification by depuration/relaying or approved processing. Perusal of availability of informations reveals that few work have been done on the aspect of bioaccumulation in green mussels in recent years (Raveendran et al., 1990; Sasikumar and Krishnamoorthy, 2010). Both the authors found that, bioaccumulation of faecal coliforms were high during monsoon and well below the threshold limit in pre-monsoon and post-monsoon seasons. But none of the work has been done on oyster. Therefore, it is important to document how varying bacterial community in different season's oysters and overlying waters. The aims of the current study was to monitoring the seasonal variation of bioaccumulation of bacterial load in the edible oyster and suggest harvest and post-harvest management practices for oyster farmers. Based on this background, we studied the bio-accumulation of bacteria (Faecal coliforms, *E. coli*, faecal *Streptococci* and Total plate count) in oysters *C. madrasensis* and growing waters of the shellfish in Ashtamudi Lake from July 2012 to June 2013.

2. Materials and methods

2.1. Study area

Ashtamudi Lake is the second largest brackish water lake in Kerala (Lat. 8°56′46.18″N and Long. 76°33′16.33″E) and has been designated as a Ramsar site based on the Ramsar Convention of Wetlands in 2002. The lake has an area of 61.4 km^2 and supports approximately 12,000 tonnes of bivalve fisheries per year. Oysters are an important edible bivalve mollusc commercially exploited from this lake. The lake is permanently connected with the Arabian Sea, and the water is exchanged daily by tides. The Kallada River flows into the lake and makes the lake brackish, which is favourable for bivalve growth. The lake is classified as mesotrophic with optimum nutrient concentration levels and good water exchange. For many bivalve species it is an important spawning and nursery ground (especially for clams) due to its sheltered condition. The bivalve fisheries and mariculture activities in this area are economically important.

2.2. Sample collection

Monthly collection of oysters (*C. madrasensis*) and harvesting waters were performed from July 2012 to June 2013. During each sampling 30 oysters and water (50 cm below the surface) from the harvesting area were collected from the commercial bivalve fishing areas of the lake. Water was collected at the surface using 500 ml sterile bottles. Water physico-chemical parameters (i.e., temperature, salinity and pH) were measured separately. The oyster and water samples were transported to the laboratory in an insulated ice-box under aseptic conditions within 4 h. The analyses were performed on the same sampling day.

2.3. Bacterial analyses

Upon arrival at the laboratory, the oysters were cleaned by scrubbing and washing under running water and drained with clean sterilized cotton. Next, the oysters were shucked with a sterile knife and the flesh and intervalvular fluid were extracted aseptically. Accumulation factors were calculated based on the method described by Burkhardt et al. (1992), where the, Bioaccumulation Factor (BAF), BAF = Co/Cw. Where, Co = the faecal coliforms concentration in oysters and Cw = the faecal coliforms concentration in waters. The bio-accumulation of faecal coliforms from the shellfish raising water of the backwater oyster was investigated from July 2012 to June 2013. Each bacterial analysis was performed in triplicate.

2.4. Faecal coliforms and E. coli

The concentrations of faecal coliforms (FC) and *E.coli* were enumerated using the multi-fermentation method (most probable number—MPN) in accordance with the American Public Health Association method (APHA, 2012). Decimal dilutions of the samples were inoculated into Lauryl Tryptose Broth (HiMedia, Mumbai, India) at 37 °C for 48 h using five tubes per dilution. All LTB tubes that showed turbidity and gas production were selected and 1 ml were transferred to tubes containing 2% Brilliant Green Lactose Broth (HiMedia, Mumbai, India) and *E. coli* broth (HiMedia, Mumbai, India). Tubes with BGLB were incubated at 35 °C for 48 h and tubes with EC were incubated in a water bath at 45.5 °C for 48 h. BGLB tubes with turbidity and gas production were quantified, and the most probable number (MPN) of coliforms per gram was determined using the MPN table for three tubes. The cultures in EC broth showing turbidity and gas production were streaked on eosin–methylene blue agar (EMB), and incubated at 35 °C for 24 h. Typical *E. coli* colonies were submitted indole, methyl red, Voges Proskauer and citrate (IMViC) biochemical tests. Faecal coliform and *E. coli* densities were estimated by computing the MPN index corresponding to the positive tube combinations. The results were expressed as MPN 100 ml^{-1} of water and MPN 100 g^{-1} of shellfish.

2.5. Total plate count and faecal Streptococci

Total plate counts (TPC) were performed using the spread plating method. Decimal dilutions of samples of homogenates were inoculated in plate count agar (HiMedia, Mumbai, India) containing 3% NaCl and examined for colony development after incubation for 48 h at 36 ± 1 °C. Colonies were counted and the data reported as colony forming units per gram (cfu/g) (Sengor, 2004; Obodai et al., 2010). Faecal *Streptococci* (FS) were counted on KF *Streptococcus* agar. The plates were incubated at 36 ± 1 °C for 48 h; dark red colonies and colonies with red and pink centres were counted as faecal *Streptococci* colonies. The results were expressed as the number of colony forming units per ml or g (Easterbook and West, 1987).

2.6. Statistical analysis

The data were expressed by seasons including Monsoon (June–Sep), Pre-monsoon (Oct–Jan) and Post-monsoon (Feb–May). All mean MPN values for TC, FC, *E. coli*, TPC (cfu/g), and FS (cfu/g) were converted to $\log_{10}$ values prior to analysis. Statistical analyses of shellfish bio-accumulation were conducted using SAS version 9.2. Analysis of variance (ANOVA) and Pearson correlation analyses were applied to determine the relationship between microbial bio-accumulation with various seasons and environmental parameters.