Effect of Depuration on Microbial Content of Mangrove Oyster (*Crassostr Ea Tulipa)* From Benya Lagoon, Ghana.

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Abstract

Mangrove oysters and water samples collected from Benya lagoon, located at Elmina in the Central Region of Ghana were investigated for microbial contamination. A total of nine fungal isolates were identified. These were Aspergilus niger, A. sulphurus, species of Penicillium, Rhizopus, Trichoderma, Fusarium, Saccharomycetes and Sterilia mycelia. Accumulations of fungal and bacterial populations in the non-depurated oysters were greater than the surrounding water. However, when mangrove oysters were depurated, populations of fungi and bacteria showed marked reduction, with some of the species disappearing completely. Saccharomycetes had a mean population of 6.3 x 10^2CFUs/g before depuration of oysters. This reduced to 0.0 CFU/g after depuration for 12 hours; and 7.0 x10^2 CFUs/g before depuration and 4.0 x 10^2 CFUs/g after depuration during the first and second investigations, respectively. Bacterial populations present on selective media (Levine Eosin Methylene Blue agar and MacConkey agar) suggested possible presence of sewage indicators, which are surrogates for water quality assay.

Key Words: Depuration, Non-depuration, Microbial content, Benya lagoon.

Introduction

Environmental pollution has become a major problem confronting many settlements, and Benya lagoon, located at Elmina, in the Central Region of Ghana is no exception. Human population has increased in the coastal town, resulting in increased domestic waste production in the area. Pollution of the environment by domestic activities, however, is the major cause of deterioration in water quality in Ghanaian lagoons (Binney, 1985).

The Benya lagoon has an influence on the socio-economic well being, and health of the people that live in the surrounding communities. The usual small scale, labour-intensive fisheries in the lagoon provides income, employment and protein for the coastal people. On the contrary, the water quality of the lagoon is compromised. This reduces the suitability of the lagoon as habitat for fish, as well as the fish quality for human consumption (Alabaster and Lloyd, 1982).

The West African mangrove oyster (*Crassostrea tulipa,* L.) is one of the popular edible marine/lagoon bivalves in Ghana. In spite of the nutritional benefits of oysters, shellfish have been identified as important vehicles of food-borne disease by virtue of their ability to concentrate viruses and bacteria from the water. The risk of illness may be particularly high, because these species are usually eaten raw or lightly cooked (Gerba, 1988).

Oysters are filter-feeders, capable of accumulating microorganisms in high concentrations. Nunes and Parsons (1998) reported that feeding oysters filter the surrounding water at a rate 2 to 5 litres/hour, eventually assimilating all the biotic and abiotic contaminants present in their environment. Bivalves such as oysters could also be used as an indicator of water quality, since these species are associated with microorganisms, which are often, generally used to indicate contamination of the water sample. One group of water and sewage indicators is the enteric bacteria - faecal coliforms, which naturally inhabit the intestines of humans and other homoiothermic animals, and are therefore, used as surrogates in water quality assay (Nester *et al.*, 2004). Wide range of bacteria can be present in wastewater and combined sewer overflows discharges, which include *Salmonella* spp., *Yersinia enterocolitica, Shigella* spp., *Enterococcus* spp., *Escherichia coli, Clostridium perfringens, Staphylococcus* spp., and *Campylobacter* spp., among others (Scott, 2002). When populations of enteric bacterium – *Escherichia coli* is excreted into the water, they die at a slower rate than pathogenic bacteria such as *Salmonella* and *Shigella*; and their presence might indicate presence of other pathogens in the water (Madigan *et al*., 2000). Bacterial selection in molluscs is related to factors such as the bacteria's adaptation to the marine environment and resistance to enzymatic degradation as well as the use of the host's gut content as a source of nutrients (Cerutti and Barbosa, 1991).

Shellfish contaminated by sewage may be cleansed of bacteria, by depuration (Fleet,

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1978). Though several water disinfection processes for depuration of contaminated shellfish are used (Richards, 1988), faecal indicator bacteria and enteric bacterial pathogens are eliminated at similar rates, but not the same for coliform bacteria and viruses (Metcalf *et al.,* 1979; Ellender and Cook, 1986; Gerba, 1988). If depuration of shellfish is properly conducted, bacteria are generally reduced to undetectable levels, while viruses are reduced at a much lower rate because they are more resistant and more persistent than bacteria (Dore and Lees, 1995).

Exposure to sewage-related pathogen in estuarine and marine environments generally occurs through two activities: consumption of raw or partially cooked shellfish and incidental ingestion of contaminated water during recreational or commercial activities, such as swimming, boating, fishing, etc. Epidemiological studies showed that symptoms of diseases associated with sewage indicators in untreated water bodies range from mild to severe gastroenteritis and dehydration to severe, extra-intestinal illness, which could be transmitted mainly through faecal-oral route (Scott, 2002). In developing countries, the disease occurs and it is most severe in children under ten years of age (Ambrus and Ambrus, 2004). This, ultimately, will affect the health of individuals who depend on the oysters as source of protein.

The present study is aimed at examining the microbial load in oysters and untreated water in the Benya lagoon, the specific microbes present, as well as the effect of cleansing of oysters by depuration.

Material and Methods

Study site

Benya lagoon, found at the western end of Elmina town was selected as the site for the investigation. The lagoon is situated along the southern coast of Central Region of Ghana between latitude $5^{o}$and 5^{o} 05^{1} N and longitudes $1^{o}30^{1}$W (see figure 1)

Sample collection

Water samples from the Benya lagoon were collected at low tide into sterile screw-capped culture tubes (20 ml), between 8.00 and 9.00 am fortnightly for three months, and held under refrigeration at 4^{o}C and quickly transported with the oyster samples to the laboratory for microbiological examinations.

Depuration of mangrove oysters

Mangrove oysters (10) were randomly sampled at each time of collection, washed thoroughly under running water, and then put into a sterile container filled with 1.5 litres of sterile sea water and depurated for 12 hours.

Examination of microbial contaminants

The shells of 10 depurated mangrove oysters were opened aseptically and their meat were homogenized. Preparation of serial dilutions were carried out by taking 10 grams of the homogenized sample (oyster meat) and transferred into 90 ml of sterile Salt Peptone Solution (SPS) as diluent, and mixed thoroughly by agitation to give 10^{-1} dilution. Further fold of serial dilution was carried out to the 10^{-2} dilution which was used for inoculation. Serial dilutions were also made for non-depurated mangrove oyster samples and water samples collected each time and later analyzed for microbial contamination.

Isolation of fungi

Yeast and filamentous fungal isolates present in depurated and non-depurated mangrove oyster samples as well as water samples were examined using pour plate technique on sterilized potato dextrose agar (PDA) medium into which sterile streptomycin (50 mg/ml) was incorporated to suppress bacterial growth. One millilitre each of the aliquot of the diluted depurated mangrove oyster sample was pipetted into 3 sterile empty, 9 cm diameter Petri dishes. About 20 ml of sterile molten medium, PDA, cooled to about 45^{o}C, was poured aseptically into the Petri dishes. Each Petri dish was covered and the content mixed thoroughly by gently swirling and moving it in a clockwise and then counter clockwise manner. Three replicates were made for each dilution. This procedure was followed for diluted non-depurated mangrove oysters and water samples. The plates were incubated at room temperature (30±2°C) for 7 days. After incubation, plates containing colonies were enumerated under illuminated colony counter, and identifications made macroscopically and microscopically using transparent tape method described by Koneman *et al*. (1979) and Singh *et al.* (1991).

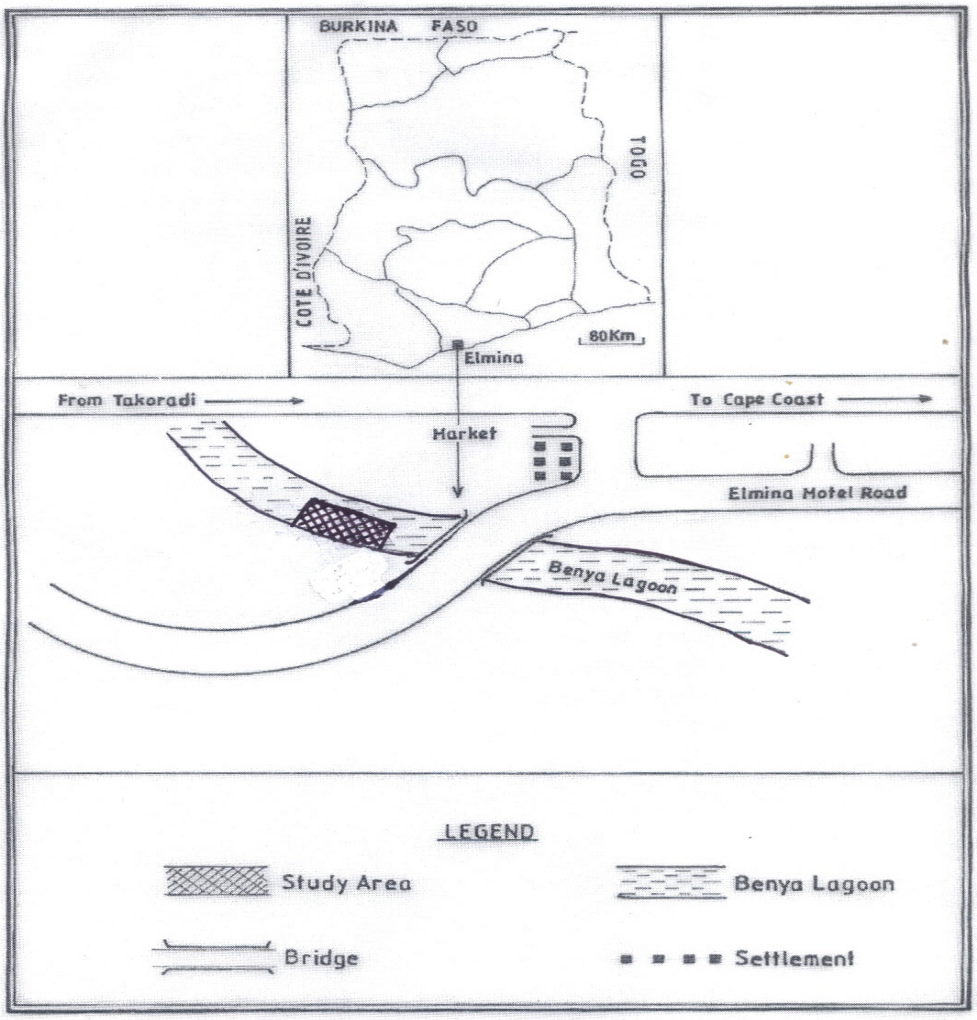

FIG.1: A SKETCH MAP SHOWING THE STUDY AREA (ELMINA HARBOUR)

Isolation of bacteria

Depurated, non-depurated oyster samples and water samples were also investigated for bacterial presence. Coliform bacteria were determined using sterile technique as outlined in plate pouring technique. Plates were made with known quantities of all the samples on a non-selective agar (Total Plate Count Agar – TPCA) and selective agars (Levine Eosin Methylene Blue Agar – LEMB; and MacConkey Agar – MA) accordingly, following the pre-incubation steps employed in the isolation of fungi. All the plates were incubated at 35°C for 48 hours. Viable aerobic plate counts were determined on TPCA to represent the bacterial load of the various samples. LEMB agar was used to enumerate the number of coliform bacteria. It serves two purposes: first, a selective medium, inhibiting the growth of gram positive bacteria, and second, as a differential medium, where colonies which ferment the lactose ("lac^+") turned purple while the *"lac"* colonies turned pink or uncoloured. MacConkey agar was also

used to enumerate coliform bacteria described by Nester *et al.* (2004). For faecal coliforms determinations, 1 ml each of the samples was poured into agar plates of selective media (LEMBA and MA) and incubated at an elevated temperature of 44.5°C for 48 hours.

Results

A total of 8 fungal isolates were obtained from the water samples, non-depurated and depurated mangrove oysters from the Benya lagoon (Table 1). The fungal isolates were *Aspergillus niger*, *A. sulphurus, Penicillium, Rhizopus* and *Fusarium* species. Other species were *Saccharomycetes* (yeast cells) *Sterilia* mycelia and *Trichomoderma* sp. *Aspergilus niger*, *Penicillium* sp. and *Sterilia* mycelia were the only fungi present in the lagoon water sample for the first week of investigation. *Aspergillus niger* recorded a mean
effect of depuration on microbial content fungal population of 3.3 x 10^2 CFU/g whereas *Penicillium* sp. and *Sterilia* mycelium, had 0.3 x 10^2 CFU/g each.

In non-depurated oysters, *Saccharomycetes* (yeast cells) had the highest mean fungal population of 6.3 x 10^2 CFU/g, followed by *Penicillium* sp. (2.7 x10^2 CFU/g), *A. niger* (2.3 x 10^2 CFU/g), *A. sulphurus* and *Rhizopus* sp (2.0 x10^2CFU/g) each, and *Sterilia* mycelia recording a value of 0.3 x 10^2 CFU/g. When the oysters were depurated, the mean fungal populations generally reduced drastically. *Saccharomycetes* (yeast cells) reduced from 6.3 x 10^2 to 0.0 CFU/g. *Penicillium* (2.7 to 0.0), *Aspergillus niger* (2.3 to 0.7 x10^2 CFU/g). The rest are *Aspergilus sulphurus*, 2.0 x 10^2 to 0.0 and *Sterilia* mycelia 0.3 to 0.0 x 10^2 CFU/g.

In the lagoon water (second week) only *Penicillium* sp, *Sterilia* mycelia, and *Aspergilus sulphurus* were represented with mean fungal population values lower than 1.0 x 10^2 CFU/g. The other fungal species were completely absent. The non-depurated oysters, again accumulated the highest mean fungal population (7.0 x 10^2 CFU/g) for *Saccharomycetes* sp.; *Trichoderma* sp. and *Sterilia mycelia* followed, with each recording 0.3 x10^2 CFUs/g. The depurated oysters revealed that *Saccharomycetes* was reduced from 7.0 x 10^2 CFU/g (before depuration) to 4.0 x 10^2 CFU/g (after depuration).

Results obtained on bacterial contaminations of lagoon water and mangrove oysters, before and after depuration on different growth media (Table 2) revealed that bacterial contamination occurred in all samples. The highest bacterial population (2.6 x 10^4 CFU/g) was recorded on the plate count agar for mangrove oysters before depuration. After depuration this reduced to 2.3 x 10^4 CFU/g. The water sample had 1.3 x 10^4 CFU/g. Water samples on LEMBA and MA recorded mean bacterial populations of 0.5 x 10^4 CFU/g each. Mean bacterial population of 2.2 x 10^4 CFU/g was recorded on LEMBA for mangrove oysters before depuration. This reduced to 2.0 x 10^4 CFU/g after depuration; and 1.8 x 10^4 CFU/g was recorded on MA for non-depurated mangrove oysters. This also reduced to 1.5 x10^4 CFU/g after depuration. The mean bacterial population on PCA for week 2 was 1.1 x 10^4 CFU/g for water sample. The mean bacterial population on LEMBA and MA was 0.4 x 10^4 CFU/g
effect of depuration on microbial content each. Bacterial contaminations of oysters before depuration on LEMBA and MA were 2.0 and 1.2 x 10^4 CFU/g, respectively. These reduced to 1.8 and 1.3 x 10^4 CFU/g, respectively.

Discussion

The general reduction in fungal population is mainly due to the depuration, since the sea water used to decontaminate the polluted oysters created an environment that did not favour proliferation of fungi. This conforms to work conducted by Richards (1988), which indicated that depuration system required a disinfection treatment of circulating water to prevent microbial build-up and recontamination of the shellfish. *Trichoderma* sp. on the other hand, remained unchanged after depuration (0.3 x 10^2 CFU.g^{-1}). This could be attributed to the fact that initial cleansing might have occurred, but because the water used for the depuration remained unchanged for the stipulated period, conditions present necessitated recontamination of fungi. *Aspergillus sulphurus* and *Fusarium*, which were absent in the mangrove oysters before depuration, prominently showed up with each recording a mean population value of 0.3 x 10^2 CFUs/g in mangrove oysters after depuration. These fungal isolates could have existed as spores or other protective forms to withstand

enzymatic action from the gut of the mangrove oysters and any adverse conditions. These fungal spores perhaps germinated by the decrease in ambient temperature during depuration, as well as the conditions of the growth medium. The recontamination could also be due to handling errors.

The presence of microorganisms in the water sample as well as the mangrove oysters suggests that microorganisms survive in the brackish water environment as well as the gut of the oysters. The results also reveal that populations of specific microbesthrive better in some environment than others due to their specific adaptations. According to Ceruti and Barbosa (1991), microorganisms selection in mollusc like oysters is related to factors such as the microorganism's adaptation to the marine environment and resistance to enzymatic degradation as well as the use of the host's gut content as a source of nutrients for their survival. However, a relatively low mean microbial population for lagoon water was observed. This therefore, supported contribution by Desmaries *et al.* (2002), to the effect that water temperature has significant effect on survival of pathogens; and lower water temperatures, generally provide more favourable conditions for microbial survival.

Bacterial colonies on MA and LEMBA showed distinct colorations. The appearance of white to colourless colonies on MA suggested the presence of non-lactose fermentors such as *Salmonella* and *Shigella* species. This is because they cannot utilize lactose but rather use the peptone in the medium to form ammonia which leads to the formation of white to colourless colonies. The red to pink colonies, also found on MA suggested the presence of coliforms such as *Escherichia, Klebsilla* and *Enterobacter* species, all of which utilize the lactose available in the medium and produced acid, which could have lowered the pH of the medium. Among the bacterial colonies present on the LEMBA was the frequently appearing one, which had a metallic green sheen colour, and was suspected to be a faecal coliform, *Escherichia coli*, since they were vigorous fermenters of lactose or sugars. Product of their metabolic activities reacted with the dyes in the medium to give the unique metallic green sheen colour. Other coliform colonies on LEMBA appeared colourless and could be *Salmonella* and *Pseudomonas* species.

Since the results in Table 2 revealed that bacterial populations in the oysters far outnumbered that of the surrounding lagoon water on both selective and the growth media, it therefore agrees with the literature in which Burkhardt III and Calci (2000) found the concentration of faecal coliforms inside the mollusc to be 4.4 times that of the surrounding water.

The study finally showed that microbial populations accumulated in shellfish (mangrove oysters) generally reduced to undetected levels under depuration. There was presence of pathogenic coliforms, in the mangrove oysters as well as the lagoon water investigated. This is likely to pose high health risk to humans who use the water and the oysters as source of protein. If it becomes necessary to use oysters, then it should be depurated.

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Table 1: Mean fungal populations of lagoon water samples, non-depurated and depurated oysters from Benya lagoon.

Fungal species	Week 1			Week 2		
	Mean fungal populations x 10^2 (CFU/g)			Mean fungal populations x 10^2 (CFU/g)		
	LW	NDO	DO	LW	NDO	DO
Aspergillus niger	3.3	2.3	0.7	0.0	0.0	0.0
Aspergillus sulphurus	0.0	2.0	0.0	0.3	0.0	0.3
Penicillium sp.	0.3	2.7	0.0	0.5	2.2	0.0
Rhizopus sp.	0.0	2.0	0.7	0.0	0.0	0.0
Trichoderma sp.	0.0	0.0	0.0	0.0	0.3	0.3
Fusarium sp.	0.0	0.0	0.3	0.0	0.0	0.3
Saccharomycetes	0.0	6.3	0.0	0.0	7.0	4.0
Sterilia mycelia	0.3	0.3	0.0	0.7	0.3	0.7

LW = Lagoon water samples; NDO = Non-depurated oyster samples;
DO = Depurated oyster samples.

Table 2: Bacterial populations of Benya lagoon water samples, non-depurated and depurated oysters on different growth media.

Growth medium	Week 1			Week 2		
	Mean bacterial population x 10^4 (CFU/g)			Mean bacterial population x 10^4 (CFU/g)		
	LW	NDO	DO	LW	NDO	DO
Plate count agar	1.3	2.6	2.3	1.1	2.4	2.2
Levine EMB agar	0.5	2.2	2.0	0.4	2.0	1.8
MacConkey agar	0.5	1.8	1.5	0.4	1.7	1.3

LW = Lagoon water samples; NDO = Non-depurated oyster samples;
DO = Depurated oyster samples.