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# Bacterial Flora of Sediment, Pond Water, Fish Feed and Fish from Fish Culture Pond of Michael Okpara University of Agriculture, Umudike, Nigeria

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**Abstract**

Samples of sediment, fish feed, pond water and tilapia *(Oreochronis niloticus)* from fish culture pond of Michael Okpara University were examined for bacterial quality. The samples were collected using sterile bottles for the sediment, pond water, fish feed samples, and a bucket for collecting the fish sample. The samples were homogenized and cultured on nutrient agar. Results revealed that the bacterial load of the fish feed was higher than that of the sediment with pond water having a lower bacterial load. Characterization and identification of bacteria using biochemical tests revealed the presence of species of organisms including *Staphylococcus saprophyticus, Enterobacter aerogenes, Escherichia coli, Micrococcus, Bacillus polymyxa, Pseudomonas fluorescens, Proteus vulgaris, Serratia marcescens, Vibrio cholerae pacini* and *Streptococcus faecalis.* Some precautionary measures were recommended such as regulating and inspecting the water quality of the pond to avoid spread of pathogens, removing an infected fish to an isolation tank which serves as a hospital for immediate treatment, application of medications, adequate stocking and fertilization of the pond.

**Key words : Fish, Fish feed, Pond water, Sediment, Bacteria.**

Many people have gone into fish culturing and this accounts for the significant availability of fish for domestic consumption as a means of employment to the coastal people as it serves as their occupation, provides raw materials for the industries giving rise to earnings and other useful purposes. Many predisposing factors have been found to be associated with infection of fish such as over crowding, accumulation of metabolic waste products particularly ammonia, organic matter in the water used for fertilization, fish feed with its high moisture content and sediment with its available nutrients (1). Other predisposing factors are injury, high water temperature and poor water quality (2). To limit the infection of fish by microorganisms, the pond should not be over-stocked to avoid over-crowding thereby spreading infection (3). Over-fertilization is also a factor that encourages the growth of microorganisms. To protect the fish culture pond from being infected, accurate measures must be taken for maintenance such as proper regulation of the water quality, removal of infected fish to an isolation tank for proper diagnosis of the ailment and proper medication. Water quality is an important aspect of aquaculture systems. Basic parameters of water quality can be grouped into four major categories : Dissolved gases, nitrogenous compounds, carbonate compounds and salinity. The significance of these parameters varies with the type of system, species and stocking density ; however, dissolved oxygen and ammonia are the two parameters of water quality that kill fish directly (1). Fishes have been observed to be associated with various diseases such as viral, bacterial, mycotic and protozoan (4). Many bacterial pathogens of such as *Aeromonas hydrophila, Pseudomonas fluorescence, Vibrio, Edwardsiella septicaemia, Aeromonas salmonicidia, Flexibacter columnaris, Mycobacterium, Norcadia* and *Flavobacterium* species have been discovered (5, 6). There is also great need to detect these organisms and eliminate them from the pond (7). Therefore this work was undertaken to establish the bacterial flora of samples sediment, pond water, fish feed and fish ; Tilapia *(Oreochromis niloticus)* from Umudike fish culture pond. Also, to determine the microbial load of the samples. Lastly, detect their relationship with fish diseases.

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## Methods

### *Source of Samples*

The test samples of pond water, sediment, fish feed and fish, tilapia *(Oreochromis niloticus)* were collected from the fish culture pond of the Michael Okpara University of Agriculture, Umudike. The feed was in the form of pellets. Pond water, sediment and feed samples were collected and stored using sterile glass containers. The fish samples were collected using a bucket.

### *Sample Preparation*

Before the samples were subjected to analysis, each of them was prepared depending on their respective nature. The body part of the fish was cut into bits with flamed scalpel (with long handle) and forceps. The cut bits were homogenized in a sterilized blender to obtain a pulpy (slurry) sample used for the analysis. Other samples, pond water and sediment were used directly.

### *Enumeration of Bacterial Load*

The total viable count was adopted following a spread plate culture technique. A unit quantity was diluted serially in sterile distilled water. Dilution was done to the sixth stage, $10^{-6}$. The dilution involved the aseptic transfer of a tenth of the test sample mixture into 9 ml of sterile water in series until the sixth step was reached. In each stage, sterile pipettes were used. Inocula were taken from the fourth and sixth diluent respectively and used for the enumeration of bacteria in the test samples.

For the sample, 1 g of the processed sample was dispensed in 9 ml of sterile water in a test tube. After mixing, 1 ml of the resulting suspension was aspetically transferred to 9 ml of sterile water in another test tube. This process was continued until the sixth diluent. The same was done for the other samples.

*Spread Plate Technique.* An innoculum of 0.1 ml of the fourth dilution was collected from each sample and was aspectically placed on a solidified sterile nutrient agar plates spread with flamed glass hockey. The innoculum was also done in triplicate and the plates were incubated at 37 C in an incubator. Incubation was for 24 to 48 hours with daily observation. When growth was established, the colonies were counted.

*Plate Counting.* Counting of bacteria was done by the use of colony counter. The instrument was switched on and its lamps were also put on. The plate to be counted was placed in the counting chamber with the light directly below it. The contact arm of the instrument was carefully lowered to rest on top of the medium supporting the culture but at a point where there was no bacteria colony, then the counting probe was tipped in ethanol, waved dry and used to touch the colonies one after the other.

With each touch, a disk was heard and a number registers in the digital counter of the instrument. For ease of counting, each plate was marked out into four

**Table 1.** Microscopy stain reactions. +ve = Positive, -ve = Negative.

| | 1st isolate | 2nd isolate | 3rd isolate | 4th isolate | 5th isolate | 6th isolate | 7th isolate | 8th isolate | 9th isolate | 10th isolate |
|---|---|---|---|---|---|---|---|---|---|---|
| Gram stain | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | +ve |
| Spore | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Flagella | -ve | -ve | +ve | +ve | -ve | -ve | -ve | +ve | -ve | -ve |
| Capsule | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Motility | -ve | -ve | +ve | +ve | -ve | +ve | +ve | +ve | -ve | -ve |
| Cell arrangement | oval cells in group | single short rods | small short rods | straight small rods | small rod singles | short single | short single | curved rods | oval cells in chains | oval cells in groups |

**Table 2.** Biochemical reaction tests. +ve = Positive, -ve = Negative.

| | 1st isolate | 2nd isolate | 3rd isolate | 4th isolate | 5th isolate | 6th isolate | 7th isolate | 8th isolate | 9th isolate | 10th isolate |
|---|---|---|---|---|---|---|---|---|---|---|
| Catalase | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve |
| Oxidase | -ve | -ve | +ve | -ve | -ve | +ve | -ve | +ve | +ve | -ve |
| Coagulase | +ve | +ve | +ve | -ve | | -ve | | +ve | ±ve | -ve |
| Indole | -ve | -ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | -ve |
| MR | +ve | +ve | +ve | +ve | -ve | +ve | -ve | +ve | -ve | -ve |
| VP | +ve | +ve | +ve | +ve | +ve | -ve | -ve | -ve | +ve | |
| $NO_3$ | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | -ve | +ve |
| $H_2S$ | -ve | +ve | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Urease | -ve | -ve | -ve | +ve | -ve | -ve | +ve | -ve | -ve | +ve |
| Citrate | -ve | +ve | +ve | -ve | -ve | -ve | +ve | +ve | -ve | |

parts of the culture plate. Counts were taken from plates supporting not less than 30 bacteria colonies and not more than three hundred. Immediately after each count, the counting probe was returned to the ethanol contained in the sterilizing container of the instrument and the plate was immediately sent for sterilization. A mean of the triplicate preparation was taken and multiplied with the appropriate dilution factor to obtain the total number of viable bacterial cells in the unit of sample analyzed. It was calculated as shown below and expressed as the number of colony forming bacterial unit per given quantity of the test sample :

$$Cfu/g = I/V \times N \times D$$

Where V = volume of sample, N = mean number of colonies counted and D = dilution factor.

*Determination of Bacteria Flora*

To determine the bacterial flora of the samples, isolates were obtained, purified and characterized using different biochemical tests and the identity of each isolate was established after incubation with an appropriate reference manual (8).

*Characterization of Bacterial Isolates.* Each bacterial colony isolated was observed closely for its

**Table 3.** Carbohydrate utilization tests of semples. +ve = Positive, -ve = Negative.

| | 1st isolate | 2nd isolate | 3rd isolate | 4th isolate | 5th isolate |
|---|---|---|---|---|---|
| Glucose | +ve | +ve | +ve | +ve | |
| Sucrose | +ve | +ve | +ve | +ve | +ve |
| Lactose | +ve | +ve | -ve | +ve | -ve |
| Maltose | +ve | +ve | +vc | +ve | +ve |
| Mannitol | +ve | +ve | -ve | -ve | +ve |
| Xylose | +ve | +ve | -ve | +ve | +ve |
| Organisms Isolated | *Staphylococcus saprophyticcus* | *Bacillus polymyxa* | Pseudomonas flourescens | *Proteus vulgaris* | *Serratia marcescene* |

**Table 3.** Continued.

| | 6th isolate | 7th isolate | 8th isolate | 9th isolate | 10th isolate |
|---|---|---|---|---|---|
| Glucose | +ve | +ve | +ve | | +ve |
| Sucrose | -ve | -ve | +ve | +ve | +ve |
| Lactose | +ve | +ve | -ve | +ve | +ve |
| MalTose | +ve | +ve | +ve | +ve | +ve |
| Mannitol | +ve | +ve | +ve | -vc | -vc |
| Xylose | -ve | -ve | -ve | | +ve |
| Organisms Isolated | *E. coli* | *Enterobacter aerogenes* | *Vibrio cholerae* | *Streptococcus faecalis* | *Micrococcus* |

**Table 4.** Total bacteiral counts of water, sediment, fishdeed and fish. N = Mean number of colonies counted, D = Dilution factor ($10^6$), V = Volume of sample (10), YV = (one tenth of the volume of sample used) = 1/10 ($10^{-1}$).

| Sample (cfu/g) | 1st | 2nd | 3rd | Mean × $10^6$ | Total count N × D × 1/V |
|---|---|---|---|---|---|
| Fishfeed (cfu/g) | 206 | 208 | 204 | 206.0 × $10^6$ | 206.0 × $10^6$ × $10^{-1}$ = 2.06 × $10^7$ |
| Pond wateri (cfu/ml) | 84 | 88 | 81 | 84.3 × $10^6$ | 84.3 × $10^6$ × $10^{-1}$ = 8.43 × $10^6$ |
| Fish (cfu/g) | 68 | 65 | 71 | 68.0 × $10^6$ | 68.0 × $10^6$ × $10^{-1}$ = 6.80 × $10^6$ |
| Sediment (cfu/ml) | 104 | 106 | 110 | 10.7 × $10^6$ | 106.7 × $10^6$ × $10^{-1}$ = 1.07 × $10^7$ |

features, which included the extent of growth, color, consistency, edge of colony elevation and presence of pigments. Biochemical characterization tests which included gram reaction, motility tests, spore capsule, flagella stainings, oxidase, catalase, coagulase, oxidase, urease, indole production, $H_2S$ production, citrate utilization, carbohydrate utilization, reduction of nitrate to nitrite, methyl red and Voges Proskaeur tests were also carried out. The cell arrangements were also obtained under the microscope. Identification followed the standard schemes (8).

## Results and Discussion

The results obtained from the analysis of the samples, are presented in Table 1 showing different reactions of the bacteria during the microscopic analysis. Some were gram positive while others were gram negative having obtained the secondary color. Some exhibited the presence of motility, spore, flagella and capsule. They also showed different cell arrangements such as oval cells in group or chains, single short rods, curved rods and straight small rods various biochemical tests used to identify the organisms are shown in Table 2. Some of the samples were positive for the tests while others were negative. The carbohydrate utilization tests of the samples with the production of acids and oxygen are shown in Table 3.

The total bacterial counts of pond water, sediment, fish feed and fish were obtained in triplicate and the total count was obtained. It was established that the fish culture pond situated at the Michael Okpara University of Agriculture, Umudike was contaminated as expected. The presence of bacterial pathogens isolated from samples such as sediment, pondwater, fish feed and fish determined using different characterization methods revealed that the fish culture pond is contaminated.

Characterization and identification of bacteria isolated revealed the presence of some bacteria such as *Staphylococcus saprophyticus, Bacillus polymyxa, Pseudomonas fluorescens, Proteus vulgaris, Serratia marcescens, Escherichia coli, Enterobacter aerogenes, Vibro cholerae* and *Streptococcus faecalis* and *Micrococcus* (Table 4). Some of these organisms are the normal flora of the pond such as *Staphylococcus saprophyticus.* Most of them are found in bird droppings which might have occurred due to the reason that the pond is an open pond such as *Serratia marcescens.* Some of them are found in the urine of man and might have occurred in the pond by man urinating near the pond.

Similar results have been obtained by previous researchers (5, 6) showing that these organisms are found in the pond. The sediment was observed to be the second sample containing high bacterial load (Table 4) although the water sample carried lower load. Observations that are similar to the pond water contributing more or less to the infection of fish have been documented (9, 10). The isolation of *Bacillus, Proteus, Pseudomonas* and *Staphylococcus* in all the samples showed that the pond was contaminated.

### *Recommendations*

The pond is a type of fresh water where fish is cultured. Many aquacultures exist nowadays due to the high economic yield of fish. Bacterial fish diseases have been found to be on the increase with over crowding, handling, accumulation of metabolic waste products and organic matter in the water (1). Different bacterial flora was isolated from samples collected at the Michael Okpara University of Agriculture fish pond showing that the pond water was contaminated. Therefore, to limit the infection of the pond water quality, which is an important aspect in

**Table 5.** Incidence (frequency) of the occurrence of Bacterial isolates from pond water, sediment, fishfeed and fish. + =Present, - = Absent.

| Bacterial species | Pond water | Sediment | Fish | Fish feed |
|---|---|---|---|---|
| *Bacillus polymyxa* | + | + | + | + |
| *Enterobacter aerogenes* | - | - | + | + |
| *Escherichia coli* | - | + | + | - |
| *Micrococcus* | - | - | - | + |
| *Proteus vulgaris* | - | + | + | + |
| *Pseudomonas flourscens* | + | + | + | + |
| *Serratia marcenscens* | + | - | - | + |
| *Syaphylococcus saprophyticus* | + | + | + | + |
| *Streptococcus faecalis* | + | + | - | + |
| *Vibrio cholerae pacini* | - | + | - | - |
| No. of genera isolated | 5 | 7 | 6 | 8 |

aquaculture system, should be well regulated. The pond should be regularly inspected for any infection to avoid spread of pathogens. Fish should be checked on regular basis and if a fish is identified as being ill, it should be removed from the pond to an isolation tank immediately, which serves as a hospital (11). When fishes get ill, they should be attended with appropriate measures (12). Medications are also recommended. Some medications used for cure have their after effect that is, they are deterimental in the long term and some can destroy the beneficial organisms (13). Instructions should be read carefully before use. Under treating the water can be just as bad as over-treating the water. Over stocking and over-fertilization of ponds by aquaculturists who crave for high yield should be stopped for the maintenance of optimum growth condition (7).

## *Conclusion*

Based on the microbial analysis of samples obtained from Michael Okpara University of Agriculture, Umudike fish culture pond, it was established that the pond was contaminated. The highest occurrence of bacterial isolates were obtained from the fish feed (Table 5) showing that it is the main source of these bacterial species in the culture system. The high bacteria must have come from deposited feed, organic manure and other sources while pond water had the lowest bacterial species showing that it contributes little to the infection of cultured fish.

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