"A study on the effect of water quality on bacterial flora of Telapia (*Oreochromis niloticus*) from two different farms"

A Thesis for the Degree of

Master of Philosophy in Nutrition

By

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DECLARATION

This is to certify that the thesis entitled **"A study on the effect of water quality on bacterial flora of Tilapia (*Oreochromis niloticus)* from two different farms "** by **Maksuda Begum** in partial fulfillmint of the requirement for the aware of **Master of Philosophy in Nutrition** has been completed under my supervision.

It is further certified that **Maksuda Begum** has fulfilled all conditions laid down in the Academic Ordinance with regard to the M.Phil course work and to the best of my knowledge; the thesis contains his original research.

30.12.09

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DECLARATION

I, hereby, humbly declare that this thesis entitled **"A study on the effect of water quality on bacterial flora of Tilapia (*Oreochromis niloticus)* from two different farms"** based on words carried by me. No part of it has been presented for higher degree.

The research work was carried out in

a) Institute of Nutrition and food Science (INFS), University of Dhaka and

b) Faculty of Biological Science, Depertment of Biochemistry and Molecular Biology, University of Dhaka .

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Dedicated
to
My beloved Parents

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Abbreviation

l	-	Liter.
ml	-	Milliliter.
g	-	Gram.
mg	-	Miligram.
CFU	-	Colony Forming Unit.
KIA	-	Kligler's Iron Agar.
MIU	-	Motility Indole Urea.
SS	-	Salmonella –Shigella agar
TCBS	-	Thio sulfate citrate bile salts sucrose agar.
E.Coil	-	Escherichio coil.
BOD	-	Biological Oxygen Demand
DO	-	Dissolved Oxygen
pH	-	Negative logarithm of H Ion concentration.

A study on the effect of water quality on bacterial flora of Telapia (*Oreochromis niloticus)* from two different farms

ABSTRACT

The effect of water quality on the micro flora of Tilapia (*Oreochromis niloticus*) reared in two different conditions, Open Water (lake) and cultured, was studied. The major objective of the present study was to compare the total viable bacterial count (TVBC), quantitatively and qualitatively, present in the mucus and intestine of both groups of fish as well as rearing water. Emphasis was given on whether there is an association of pathogenic bacteria with the fish and rearing water or not. Two groups of fishes each consisted of five fish samples each were collected from Gazipure Farm (cultured fish) and Dhanmondi lake (Open Water fish) during the month of August to November' 2006. Open Water fish did not receive any special feed but cultured fish were reared with selected fish feed. In the present study the quality of the fish feed and their microbial count were also observed. Two different types of fish feed samples powder form (FF_1) and pellet form (FF_2), were collected from locally available sources at Gazipure. For the isolation of bacterial strains Nutrient, MacConkey, SS and TCBS agar media were used and the estimation of the total viable bacterial count (TVBC) was done by streak and spread plate method. Identification of the bacterial strain was done by cultural, morphological and biochemical tests. On Nutrient agar media, the TVBC of intestine and mucus of Open Water Tilapia fish and rearing water of Open Water (lake) were 1.85 x 10^9, 9.9x10^5 cfu/g , and 1.75 x 10^5 cfu/ml, respectively. The TVBC on the same media for intestine and mucus of cultured Tilapia fish and rearing water of pond were 4.96 x 10^6, 1.29 x10^5 cfu/g , and 1.4 x 10^4 cfu*/ml,* respectively. Similarly, TVBC on MacConkey agar media for the water and intestine and mucus of the fish reared in Open Water (lake) were much higher (0.96 x 10^5 cfu/ml, 1.73 x 10^8 and 1.48 x 10^5 cfu*/g,* respectively) than

cultured varieties reared under controlled condition (1.20 x 10^4 cfu/ml , 3. 90 x 10^6 and 7.03 x 10^4 cfu/g, respectively). 10 different types of Gram negative bacteria were isolated from the intestine and the mucus of the Open Water fish compare to only 6 types from the intestine and 7 types from the mucus of cultured variety. The TVBC in FF_1(fish feed 1) grown on Nutrient agar media and MacConkey agar media were 1.75 x10^9 and 1.65x10^8cfu/g , respectively whereas in case of FF_2 (fish feed 2), it were 1.1 x 10^8 and 2.15 x 10^8cfu/g . Identification and characterization through cultural, morphological and biochemical tests showed that the frequently encountered bacteria in the samples of both Open Water and cultured fish as well as in the rearing water and fish feeds (FF_1 and FF_2) were *Aeromonas, Plesiomonas, Escherichia coli, Pseudomonas, Salmonella, Shigella,* and *Klebsiella*. The bacteria were predominantly Gram negative rods (91%). Significant finding were that *Salmonella, Shigella,* and *Vibrio spp.* were present only in the samples collected form Open Water (lake) environment. Furthermore, the physical, chemical and biological parameters of the pond water were far more acceptable than those of the Open Water (lake). The rearing water of the Open Water (Dhanmondi lake) had an average P^H, BOD and DO values of 6.92, 35.8 ppm and 5.76 ppm respectively were as for pond water those were 7.24, 1.97 ppm and 6.94 ppm respectively. The presence of pathogenic bacteria and high level of organic contaminations in the Open Water (Dhanmondi lake) as reflected by higher BOD render it unsafe and unsuitable for consumption or handling of fish reared in this environment. Fish reared in this environment may cause severe health hazards which may lead to severe problems in general public heaths. Thus, it can be recommended that by maintaining good quality of rearing water as well as fish feed contamination and prevalence of deadly pathogenic bacteria can be minimized in the fish body.

Chepter-1

- Introduction
- Objective of the Study

1. Introduction

In a revering country like Bangladesh, the role of fishery is very important. Bangladesh is rich in fisheries sector. This sector contributes about 5.4% of GDP in 1998[1]. In Bangladesh numerous rivers and their tributaries in addition to lakes canals, ponds, marshes, cultural ponds, hatcheries etc constitutes a large fresh and cultural water body that harbors about 257 species of fish under 144 genera and 56 families[2]. Freshwater aquaculture can make a significant contribution to bridging the widening gap between demand for and supplies of fishery products in Asia, in the face of declining capture fisheries production and growing populations.

In all countries, water pollution due to discharge of industrial pollutants needs to be minimized if it cannot be stopped. Water use conflicts between crops and aquaculture should be minimized as far as possible. Fertilizers and agro-chemicals should be used judiciously on crops to protect the natural habitats of fish. Research on fish nutrition should be undertaken in order to develop cheap but good quality feed. Fish as food has been playing an important role in the world food security including Bangladesh; Fish contribute about 80% of the total animal protein in the diet of Bangladesh[2].

Fish consumption preferences vary across countries, but in general (a) higher-income groups consume more fish, though the proportion of the food budget allocated to fish expenditure is higher among low-income groups; (b) rural people consume more than urban dwellers; (c) fish producers in general consume more fish than non-producers; and (d) demand for fish is very sensitive to price changes. Religious beliefs and ethnic and geographical differences also explain variations in fish consumption across countries. Fishes supply essential

nutrients needed by our body i.e. protein, fat vitamins, minerals and energy. (FAO).

More than 80 percent of the animal protein in the Bangladeshi diet comes from fish. An ounce a day has been shown to cut risk of heart attacks by 50 percent. The omega-3 oil in fish can relieve symptoms of rheumatoid arthritis, osteoarthritis, asthma, psoriasis, high blood pressure, Raynaud's disease, migraine headaches, ulcerative celitis and, possibly, multiple sclerosis. Fish intake raises good type HDL cholesterol and lowers triglycerides. It guards against glucose intolerance and Type 2 diabetes.Some fishes exhibits anti-cancer activity especially in blocking development of colon cancer and spread of breast cancer Fish highest in omega-3 fatty acids include sardines, **tilapia**, herring, salmon, tuna. Fish fatty acids linked to mature brain development in infants and omega-3 intake may protect against Alzheimer's. Fish amino acids may reverse smoking damage and infarction. It has effect on cancer administration, patients. It also has effect on joints as treatment of rheumatoid arthritis with omega-3 fatty acids is a well established method.

Tilapia has become the third most important fish in aquaculture after carps and salmonids, with production reaching 1,505,804 metric tons in 2002[3]. Because of their large size, rapid growth, and palatability, a number of tilapiine cichlids are at the focus of major aquaculture efforts, specifically various species of *Oreochromis*, *Sarotherodon*, and *Tilapia*, collectively known colloquially as tilapias. Like other large fish, they are a good source of protein and a popular target for artisanal and commercial fisheries. Originally, the majority of such fisheries were in Africa, but accidental and deliberate introductions of tilapia into freshwater lakes in Asia have led to outdoor aqua culturing projects in countries with a tropical climate such as Papua New Guinea, the Philippines, and

Indonesia[1]. In temperate zone localities, tilapiine farming operations require energy to warm the water to the tropical temperatures these fish require. One method involves warming the water using waste heat from factories and power stations.[4]

Tilapiines are also among the easiest and most profitable fish to farm. This is due to their omnivorous diet, mode of reproduction (the fry do not pass through a planktonic phase), tolerance of high stocking density, and rapid growth. In some regions the fish can be put out in the rice fields when rice is planted, and will have grown to edible size (12–15 cm, 5–6 inches) when the rice is ready for harvest. One recent estimate for the FAO puts annual production of tilapia at about 1.5 million tonnes, a quantity comparable to the annual production of farmed salmon and trout[4]. Unlike salmon, which rely on high-protein feeds based on fish or meat, commercially important tilapiine species eat a vegetable or cereal based diet. Tilapias raised in inland tanks or channels are considered safe for the environment, since their waste and disease should be contained and not spread to the Open Water (lake).[5]

Set against their value as food, tilapiines have acquired notoriety as being among the most serious invasive species in many subtropical and tropical parts of the world. For example *Oreochromis niloticus*, *Oreochromis mossambicus*, *Sarotherodon melanotheron melanotheron*, *Tilapia mariae*, and *Tilapia zilli* have all become established in the southern United States, particularly in Florida and Texas.[6]

The Nile Tilapia, *Oreochromis niloticus* is a cichlid fish of African origin which is native from Sudan into east Africa through the Congo to Liberia. It is also commercially known as Mango fish or Nilotica[7]

***Oreochromis niloticus*:**

Nile Tilapia	
	
Scientific classification	
Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Perciformes
Family:	Cichlidae
Subfamily:	Pseudocrenilabrinae
Tribe:	Tilapiini
Genus:	*Oreochromis*
Species:	***O. niloticus***
Binomial name	
Oreochromis niloticus	

In modern aquaculture, Open Water (lake)-type Nile tilapia are not too often seen, as their flesh has a dark color that is not much desired by many customers, and because it has a bit of a reputation of being a "trash fish" associated with poverty[8]. On the other hand, they are fast-growing and give good fillets; leucistic ("Red") breeds which have lighter meat have been developed and these are very popular. Hybrid stock is also used in aquaculture; Nile × Blue Tilapia hybrids are usually rather dark, but a light-colored hybrid breed known as "Rocky Mountain White" tilapia is often grown due to its very light flesh and tolerance of low temperatures[9]. The Nile Tilapia has recently been discovered in a small

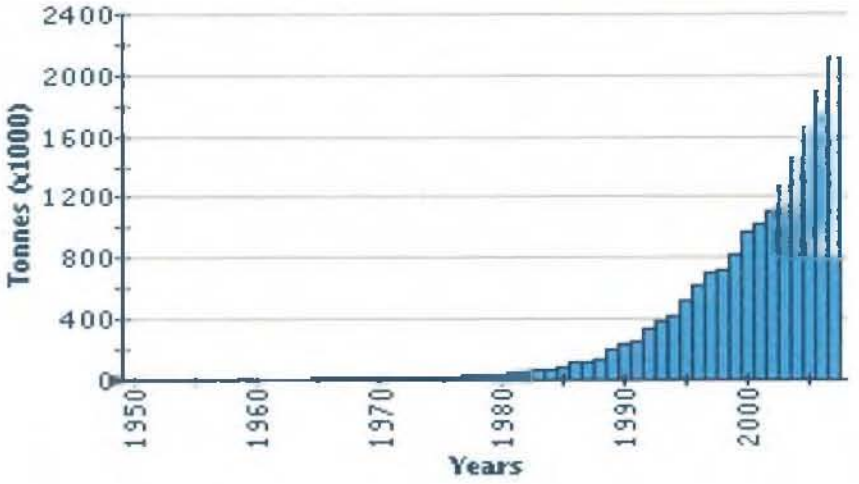
stream in central Arkansas. This invasive species may harm the other aquatic life present in this stream within the next few years, depending on quickly it is able to reproduce and how adapted it is to competition with other aquatic vertebrates. As of yet, there is evidence to support the possibility that the Nile Tilapia has established a strong breeding ground and will eventually endanger other fish species, possibly competitively exclude them.

Nile tilapia occurred during the 1960s up to the 1980s. Nile tilapias from Japan were introduced to Thailand in 1965, and from Thailand they were sent to the Philippines. Nile tilapias from Cote d'Ivoire were introduced to Brazil in 1971, and from Brazil they were sent to the United States in 1974. In 1978, Nile tilapia was introduced to China, which leads the world in tilapia production and consistently produced more than half of the global production in every year from 1992 to 2003. The uncontrolled breeding of tilapia in ponds, which led to excessive recruitment, stunting and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish. The development of hormonal sex-reversal techniques in the 1970s represented a major breakthrough that allowed male monosex populations to be raised to uniform, marketable sizes. In addition, research on nutrition and culture systems, along with market development and processing advances, led to rapid expansion of the industry since the mid 1980s. Several species of tilapia are cultured commercially, but Nile tilapia is the predominant cultured species worldwide.

Main producer countries of Oreochromis niloticus (FAO Fishery Statistics, 2006)

Global aquaculture production of Oreochromis niloticus

(FAO Fishery Statistic)



China is by far the largest producer of farmed Nile tilapia. By 2003 annual Chinese production had risen to nearly 806 000 tonnes and Egypt reported a production of nearly 200 000 tonnes in that year, while the Philippines, Thailand and Indonesia produced 111 000 tonnes, 97 000 tonnes and 72 000 tonnes respectively. The other five 'top ten' Nile tilapia producers were the Lao People's Democratic Republic, Costa Rica, Ecuador, Colombia and Honduras. Brazil and Taiwan Province of China are also major producers of Nile tilapia and many others, such as Cuba, Israel, Malaysia, the USA, Viet Nam and Zimbabwe produce significant quantities annually. However, the production of these countries is reported to FAO under the general statistical categories 'tilapias nei' (which may include other tilapia species) and 'freshwater fishes nei'. It is therefore not possible to include their production data in the above chart until the reporting countries refine their statistical returns to FAO; for the same reason these production locations are not shown in the aquaculture geographical distribution section of this fact sheet.

Aquaculture Microbiology:

In aquaculture the micro flora and their various fish and invertebrate hosts share ecosystem, and become easily colonized by bacteria. This intimate relationship may result in disease epizootics or commensally relationships. Aquaculture will depend on extensive understanding of the complex interactions between the cultured organisms and bacterial communities which develop on mucosal surfaces and in the rearing systems. Some of the more common bacterial pathogens found in freshwater fish are mentioned here. Such as *Aeromonas spp, Pseudomonas spp, Azobacter, Escherichia coli, Shigella, Salmonenella, Vibrio spp, Klebsiella* etc.

Diseases And Control Measures

Diseases can often be avoided by maintaining a high quality environment and reducing handling stress. The major disease problems affecting Nile tilapia are included in the table below. In some cases antibiotics and other pharmaceuticals have been used in treatment but their inclusion in this table does not imply an FAO recommendation.

DISEASE	AGENT	TYPE	SYNDROME	MEASURES
Motile Aeromonas Septicaemia (MAS)	Aeromonas hydrophila & related species	Bacteria	Loss of equilibrium; lethargic swimming; gasping at surface; haemorrhaged or inflamed fins & skin; bulging eyes; opaque corneas; swollen abdomen containing cloudy or bloody fluid; chronic with low daily mortality	KMnO4 at 2-4 mg/litre indefinite immersion or 4-10 mg/litre for 1 hour; antibiotics (need 'extra-label use permit' in the USA), e.g. Terramycin® in feed at 50 mg/kg fish/d for 12-14 d, 21 d withdrawal
Vibriosis	Vibrio anguillarum &	Bacteria	Same as MAS; caused by stress & poor	Antibiotic in feed

	other species		water quality	
Columnaris	Flavobacterium columnare	Bacterium	Frayed fins &/or irregular whitish to grey patches on skin &/or fins; pale, necrotic lesions on gills	KMnO4 as with MAS; indefinite immersion with CuSO4 at 0.5-3 mg/litre, depending on alkalinity
Edwardsiellosis	Edwardsiella tarda	Bacterium	Few external symptoms; bloody fluid in body cavity; pale, mottled liver; swollen, dark red spleen; swollen, soft kidney	Antibiotic in feed
Streptococcosis	Streptococcus iniae & Enterococcus sp.	Bacteria	Lethargic, erratic swimming; dark skin pigmentation; exophthalmia with opacity & haemorrhage in eye; abdominal distension; diffused haemorrhaging in operculum, around mouth, anus & base of fins; enlarged, nearly black spleen; high mortality.	Antibiotic in feed, e.g. Erythromycin at 50 mg/kg fish/d for 12 d (requires 'extra-label use' permit in the USA)
Saprolegniosis	Saprolegnia parasitica	Fungus	Lethargic swimming; white, grey or brown colonies that resemble tufts of cotton; open lesions in muscle	KMnO4 or CuSO4 treatments; use 1 mg/litre of CuSO4 for every 100 mg/litre alkalinity up to 3.0 mg/litre CuSO4; formalin at 25 mg/litre indefinite immersion or 150 mg/litre for 1 h
Ciliates	Ichthyophthirius multifiliis; Trichodina & others	Protozoan parasite	Occurs on gills or skin	KMnO4, CuSO4 or formalin treatments
Monogenetic trematodes	Dactylogyrus spp.; Gyrodactylus spp.	Protozoan parasite	Occurs on body surface, fins or gills	Same as for ciliates

Cultured Vs. Open Water (lake) Fishes:

As Bauer (in Dogiel et al. 1970) points out, there are several major differences, positive and negative, between pond-cultured and non-pond cultured fishes, their disease control and prevention. These differences may be explained by consideration of the design of culture systems vs. Open Water (lake) environments.

I) Density: Due to the proximity of co-specifics, transmission of parasites is facilitated in cultured pond.

II) Confinement: The uniformity of a culture environment in terms of temperature, pH, oxygen concentration, food availability, and other physical-chemical-nutritional factors allows little latitude for cultured fishes to select more suitable conditions.

III) Monoculture: The cultivation of one or perhaps a few species in a system that is periodically sterilized.

IV) General stress: Disease in fishes can be physiological response to stress (Gratzek, 1975). Some common sources of stress are transportation, excessive handling, abrupt temperature fluctuations, physical-chemical factors, and overdosing with medicants. Many fishes die outright from severe stress but many more often from chronic less-severe stresses. Many fish pathologists feel that fish carry bacteria and protozoan in low numbers in their blood and kidney tissues as well as on the surface of their skin and intestines, but remain healthy until stressed. Fish in intensive culture are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, drug treatments, undernourishment, fluctuating temperatures, and poor water quality. All of these factors can impose considerable stress on the homeostatic mechanisms of fish rendering them susceptible to a wide variety of pathogens.

Another major issue pertaining to fish health is the concern over the introduction of infectious diseases, particularly those that are not endemic to the area, through

fish culture facilities to Open Water (lake) fish populations. Open Water (lake) fish stocks can become infected and suffer high mortalities through the release of pathogens in untreated hatchery effluent or through the stocking or escapement of diseased fish into aquatic systems. (Leeds, 2004).

Fish Culture:

Water bodies for aquaculture are largely owner-operated in Bangladesh. Polyculture, monoculture, cage culture and rice-fish farming are now a day practiced in all over the world. Bangladesh has major (Indian) carp (rohi, katla and mrigal), tilapia and silver carp as the dominant species. Cultured ponds in Bangladesh, use of relatively lower inputs and thus are semi-intensive or improved extensive. Fish has become an increasingly important source of protein in Bangladesh, with the annual rate of per caput fish consumption generally increasing. Bangladesh aims to increase fish production, improve export earnings, provide more animal protein and expand employment opportunities. Now a day, the practice of culture fishes is well established. Tilapia fish is a very rapid growing fish. (FAO report).

Pond Management:

A modern cultural pond must have to maintain a safer aquatic zone for the production of healthy fishes. It is recommended to fulfill some of the basic water quality properties to raise up fishes in cultural pond with good health. ("Pond Management" GenoMar Supreme Tilapia^{TM} Inc. Norway, 2005).

Parameters	Normal range	Unit
Transparency	25-40	c.m.
Temperature	28-32	c
Dissolved oxygen	Greater than 5	ppm
p^H	6.5-8.8	-----
Alkalinity	90-250	g/L
Ammonia	less than 0.3	g/L
Nitride	less than 10	
Phosphate	less than 0.025	
Hardness	60-150	g/L

Parameters are shown below with their acceptable ranges.

In order to fix up the above reference in practical the following steps should be taken.

1. p^H of the pond should be taken twice in a week. If p^H of the water increases the lime should be added to lower the p^H.

2. Temperature should be counted daily.

3. DO (Dissolved Oxygen) should be measured in every day and night. If necessary airiator can be used.

4. To sweep up the condensed gas that stored under ground water level, it is recommended to us a propeller which will not allow to store the gas and dragged the stored gas out.

Fish Feeds:

Since the middle of 1980s, the increasing use of feeds has been the main method of increasing production for all the finfish species. Prepared feeds that provide a complete diet (adequate protein, lipids, carbohydrates, vitamins and minerals) are readily available in developed countries and are also manufactured and available in developing countries with an export market for high quality tilapia products.

Trash fish provide the major feed type for finfish culture. Until a few years ago, almost all fish farmers used entirely fresh feed, ground or minced, consisting of a variety of fish and non edible part of fish, right after catch or in frozen state. The inclusion of compound feed, in dry state, was 2% in 1988, and 1993 it was 9% with 91% of raw fish (mostly frozen, in wet state), and now more compound feed is used. Meanwhile, the uses of formulated feeds are greatly expanded for freshwater and marine fish species recently. Most pellets containing higher amount of compound feed keep much higher water stability. Now a day, in fish feed various food components are used. Vitamins, minerals, protein and carbohydrate are among the major components of the fish feed. Wheat, rice, soybean, calcium, flour and micronutrients like iron, iodine, sulphur, phosphorus are used in fish feed as additives[9].

Present Situation Of Lakes In Dhaka City:

The water of the Gulsan-Baridhara is the most polluted among the city's five lakes according to a study conducted by the department of Environment (DoE). The study of the lake's water revealed that presence of bacteria was 1200 counts, which researchers say is not even fit to be touched by humans. The tolerable bacteria count in water bodies is 200 or less. According to the research conducted at the Bangladesh Atomic Energy Commission (BAEC), marine life could not survive in water that had a Dissolved Oxygen (DO) level of less than 5.0. The Biological Oxygen Demand (BOD) in Gulsan-Baridhara lake was found at 35, indicating serious contamination of the water. The acceptable level is three or less. Hundreds of drains carry toxic waste and untreated sewage in the Gulsan-Baridhara Lake every day. The DoE study in the effect solved the mystery of dead fishes in the Dhanmondi lake a couple years ago. Water of Dhanmondi lake, part of which was excavated and cleaned about 6 years ago, had the most ideal BOD level with 1.9 counts. It however has 600 counts of coliform, 6.1 counts of DO and 6.95 counts of pH. With 9202 counts, the water of the Cresent Lake has the second

highest count of coliform. At 8.3 it has the best DO level among the five lakes. Cresent lake's BOD level (2.1) is the second best after Dhanmondi lake. The water of Ramna lake is the most pollutant behind the Gulshan-Baridhara lake. It has 700 counts of coliform. 1.3 counts of DO, 25 counts of BOD and 6.52 counts of pH. The Sitadal Lake in Baridhara has 6.91 counts of pH, 2.6 counts of BOD, 6.6 counts of DO, and 500 counts of oviform. This is the common picture of our inland water system which is much polluted and has adverse effect on the aquatic life like fishes. As well as on general public health as huge amount of fishes are being caught and consumed by people of the lower income group. In that contrast cultured fishes are grown is much healthy environments. (Dept. of Environment, 2004, Daily Star, 29th December, 2004).

Objectives of the study

General objective:

To see the effect of water quality on microbial flora of cultured(pond) and open water(wild) Tilapia*(Oreochromis niloticus)*fish.

Specific objectives:

1. To isolate bacteria from small intestine, mucus of tilapia *(Oreochromis niloticus)* fish as well as form rearing waters and fish feeds.
2. To calculate the total viable bacterial count on Nutrient and MacConkey agar.
3. To characterize the isolates on Nutrient, MacConkey, SS, and TCBS agar media by cultural and morphological analysis.
4. To identify isolates by biochemical tests.
5. To measure of the physiochemical parameters such as BOD, DO, $P^{H.}$ and temperature of the rearing waters both pond and open water (lake).

Chepter-2

- Review of Literature

2. Review Of Literature

Most of the tilapia production in Bangladesh comes from aquaculture which largely depends to greater extent on aquatic environment. Water quality is the main factor that determines degree of production. Contaminated water is not suitable for aquaculture. Contamination may result from rupturing fish intestine during poor processing or inadequate washing. It has been suggested that intestinal micro flora is the causative agent for food spoilage. Bacterial activity, so far is the most important factor influencing fish quality, so bacterial numbers can be used as an index of quality. Fish possess bacterial populations on or in their skin, gills, and light-emitting organs. In addition, the internal organs (kidney, liver, and spleen) of healthy fish may contain bacterial. The numbers and taxonomic composition of the bacterial populations often reflect those of the surrounding water [10] . Various studies from different parts of the world have demonstrated that many enteric organisms are associated with fish and initiate various fish-borne diseases in man.

Ahmed H. Al-Habra & Naim Uddin showed that The bacterial flora occurring in brackish pond water, sediment, gills and intestine of healthy tilapia cultured in Saudi Arabia were estimated both quantitatively and qualitatively, and the isolates were identified to genus or species level. Total viable count of bacteria ranged from $1.4\pm1.5\times10^{3}$ to $8.6\pm2.7\times10^{3}$ cfu ml^{-1}; $1.2\pm3.1\times10^{6}$ to $7.3\pm1.1\times10^{7}$ cfu g^{-1}; $8.7\pm1.9\times10^{5}$ to $2.1\pm0.9\times10^{6}$ cfu g^{-1}; and $2.8\pm2.4\times10^{7}$ to $1.0\pm1.6\times10^{8}$ cfu g^{-1} in the pond water, sediment, gills and intestine of brackish water tilapia, respectively. In total, 19 bacterial species were identified. The bacteria were predominantly Gram-negative rods (87%). Pond water and sediment bacteria influenced the bacterial composition of gills and intestine of tilapia. In contrast to gill bacteria, more diversification was observed in intestinal bacteria. The predominant (prevalence >10%) bacterial species were *Vibrio parahaemolyticus, Vibrio carchariae, Vibrio alginolyticus, Chryseomonas* sp., *Vibrio vulnificus*, and *Streptococcus* sp. in all the

populations with the exception of the sediment population where *Streptococcus* sp. was replaced by *Shewanella putrefaciens*. *Vibrio* spp. (58% of the total isolates) dominated the total bacterial population[11].

In another study Ahmed H. Al-Habra & Naim Uddin showed that seasonal quantitative and qualitative analyses of the bacterial flora associated with the intestine of hybrid tilapia (*Oreochromis niloticus*×*Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia were carried out. The isolates were being identified to genus or species level. Total viable counts (TVC) of bacteria in the intestine varied between $6.8\pm1.9\times10^6$ to $7.5\pm1.4\times10^7$ colony forming units (cfu) g^{-1} in early summer, $1.6\pm2.0\times10^6$ to $5.1\pm2.5\times10^7$ cfu g^{-1} in summer, $3.1\pm1.4\times10^8$ to $1.3\pm2.2\times10^9$ cfu g^{-1} in autumn, and $8.9\pm1.8\times10^5$ to $1.3\pm0.9\times10^7$ cfu g^{-1} in winter. Altogether, 17 bacterial genera were identified from the intestine of tilapia. The bacteria were predominantly Gram-negative rods (77%). *Aeromonas hydrophila*, *Shewanella putrefaciens*, *Corynebacterium urealyticum*, *Escherichia coli* and *Vibrio cholerae* were the most abundant species with a prevalence of>10% in most cases except *V. cholerae*. Considerable numbers of *Pseudomonas* spp. were found only in winter. *Photobacterium damselae*, *Pasteurella* spp., *Cellulomonus* sp. and *Bacillus* sp. were present in some seasons of the year[12].

Ojala (Ojala, 1968) and Oliver (Oliver et. al, 1980) isolated *Aeromonas spp*, from healthy and moribund fish[13, 14].

Species of *E.coli* are generally found in the gastrointestinal tract of homoeothermic species but were not found in significant number in the fish (Wood et. al, 1972). So presence of *E.coli* in the fish can probably be directly related to the fecal contamination of water with worm blooded animal and the fish are probably the transient carriers of the organisms, unhygienic handling and processing of the fish undoubtedly induce the *E.coli* as secondary bacterial contamination.

In 1977 Boulanger *et al.* isolated *Aeromonas hydrophila* and *Aeromonas sorbia* from fish and most of these *Aeromonas* were found pathogenic.

Vibrio alginolyticus has been found in water in widely diverse geographic locations. *Vibrio alginolyticus* has been etiologically associated with gastroentities, cellulitis, acute otitis media, conjunctivitis, wound infections and these infections have generally been reported in fishermen, healthy swimmers and fish cutters[15].

Vibrio minicus, a newly described organism has been isolated in Bangladesh from patient with diarrhoea and from fresh water samples[16, 17]. Vibrio *minicus* has also been isolated from fish in Japan.[18]

Vibrio cholerae non-01 has been isolated from fresh water and estuarine water throughout the world as well as from fish.[17,19] *Vibrio cholerae nimicus* has been isolated only with sporadic cases of dairrhocal diseases.[16]

Gennari M, 1988 reported that *Pseudomonadaceae, Neisseriaceae, Flavobacterium, Cytophaga, Enterobacteriaceae, Coryneform bacteria* and *Micrococaceae* were the most common bacteria in fresh fish.

Zheng et al. collected samples of epidermis, gills and digestive tract of dead Pagrosomus major in China. The genera of the bacteria isolated from water and fish samples were *Vibrio, Pseudomonas, Aeromonas,* and *Flavobacterium* [20].

Toranzo et al. studied in the micro flora associated with healthy and diseased Turbot *(Scophthalmus Maximus)* from three farms in northwest Spain. *Vibrio* spand *Peudomonas spp* were present in both types of samples.[21]

Sung-Hunghung et al. isolated Citrobacter freundil, *Pseudomonas spp, Aeromonas spp*, from gaint fresh water prawn.[22]

Starliper et al isolated *motile Aeromonas spp.* from fresh water bivalves from the Ohio river and the mean total bacteria counts were $1.6x10^6$ CFU/g.[23]

In India, Das and Mukherjee measured micro flora of 240 Indian major carp Rohu (*Labeo rohita)* at fry and fingerling stages. *Aeromonas hydrophila,* bacillus sp and *Pseudomonas mattophilia* were the most freqeutnly encountered bacterial. Other species isolated were *Enterobacter agglomerans, Yersinia pseudotuberculosis, Klebsiella ozaenae.*[24] Lida et al. isolated bacterial flora from the digestive tract of cultured Pacific oyster[25].

Austin B.showed that fish possess bacterial populations on or in their skin, gills, digestive tract, and light-emitting organs and that numbers and taxonomic composition of the bacterial populations often reflect those of the surrounding water.[26]

Recently Al-Harbi AH, 2005 has worked on microbiological quality changes in the intestine of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus)* in fresh and frozen storage conditions.[27]

Shankar Chandra Mandal investigated the abundance of coliform bacteria in Nile tilapia sampled from different sources. Densities of total aerobic bacteria, total coliform (TC), faecal coliform, (FC) and *Escherichia coli* were measured from different organs of Nile tilapia sampled from pond, gher and market using serial dilution and spread plate techniques. Significant differences were observed in various parameters of bacterial density between and within different organs of Nile tilapia ($p<0.05$). Significantly higher density of faecal coliform was detected in the muscle, gill and intestine in Nile tilapia sampled from pond than that of market. The highest density of total aerobic bacteria ($8.81 \pm 0.45 \times 10^5$ cfu/g), TC ($3.00 \pm 0.20 \times 10^4$ cfu/g) and *Escherichia coli* ($1.45 \pm 0.19 \times 10^3$ cfu/g) were measured in the intestine and FC ($3.00 \pm 0.67 \times 10^3$ cfu/g) in the gill of Nile tilapia sampled from market. Findings of the present study suggest that Nile tilapia may be faecally contaminated during culture period, storage and transportation and unhygienic marketing.

In Bangladesh very limited numbers of studies were done on the isolation of *Aeromonas from* fish. In one study all the specimens taken from 20 fresh water prawns collected from Dhaka city markets were positive for *Aeromonas,* while in two other studies swabs taken superficial skin ulcers were positive for *Aeromonas* in 8 out of 15(53.3%) fresh water fish and 11 out of 15 (73.3%) brackish water fish,[28,29]

Ahmed Shakil, M.Sc Thesis, INFS, DU, 2000 studied on the qualitative and quantitative analysis of bacterial flora associated with mucus, intestine, kidney and gill of fresh water fish in Dhaka city.

In Bangladesh, Banu *et al.* (2001) investigated the bacterial load in pond water. They observed that the mean bacterial load in surface water varied from 1.39x 10^5 (July 94) to 3.11x10^7 CFU/ml (September 93), while that of bottom water ranged from 1.0x 10^6 (May 94) to 5.90x 10^7 CFU/ml (October 93).[30] Romanenko (1971) reported that the bacterial number in reservoir water was 1.43-0.18x 10^6 ml of water[31]. Tewary and Mishra (1985) reported that fresh water bacteria varied from 1 to 3.00x 10^3 CFU/ml in the lake water[32]. Araki and Kitamikadi (1978) pointed out that the population density of bacteria ranged from 0.0 to 1.8x10^5 cell/ml of water in some river and pond water of Japan[33].

Rahman *et al.* (2001) observed the virulence of viable but non culturable state (VBNC) of *Aeromonas hydrophila* in a carp fish *Carassius auratus* and recorded the LD50 values of 106.18 CFU/fish, 108.45 CFU/fish and >109.11 cell/fish in VBNS cells. A perusal of the data shows that the natural average bacterial load of 3.3x10^6 CFU/ml or below do not produce any significant mortality in *Oreochromis mossambicus*[34].

A study was conducted in the local tilapia industry to determine the microbial quality of pond water, prevalence of bacterial pathogens and their anti-microbial resistance using the disk diffusion method. Seventy-five apparently healthy fish and 15 pond water samples from three of the four commercial tilapia fish farms in

the country were processed. The 202 bacterial isolates recovered from fish slurry and 88 from water, belonged to 13 and 16 genera respectively. The predominant bacteria from fish slurry were *Pseudomonas* spp. (60.0%), *Aeromonas* spp. (44.0%), *Plesiomonas* (41.3%) and *Chromobacterium* (36.0%) ($P < 0.05$; v2) compared with isolates from pond water where *Bacillus spp.* (80.0%), *Staphylococcus* spp., *Alcaligenes* spp. and *Aeromonas spp.* (60.0%) were most prevalent ($P < 0.05$; v2).

Chepter-3:

- Methods and Materials

3. Methods and materials

To carry out the qualitative and quantitative analysis of bacterial flora associated with the mucus and intestine of healthy Open Water (Lake) and cultured(Pond) Tilapia fishes, there rearing water and the Fish Feeds ,the following methods were used as followed.

3.1 Sample: All the samples of the fish belong to the genus *Tilapia* and species *niloticus*. Water was sampled from the same rearing pond and Open Water (lake).

3.2 Place Of Sample Collection: All the samples fish and water were collected from Dhanmondi Lake and Gazipure Farm of Dhaka division.

3.3 Period Of Collection: The samples were collected in August to November 2006. The collected time was between 6.30 to 7.30 am.

3.4 Methods For Sample Collection: During collection, fish samples were examined carefully. Fishes with diseases, mechanical injuries during catching were carefully sorted out. Fish were caught and kept in a tank of 30 liter capacity. Rearing water was collected in special air tight sterile tubes. Three water samples were collected from three different locations of the same rearing lake .Water samples were collected with special care and followed the following steps:

1. Putted on gloves and goggles. To avoid contamination, thoroughly rinse the water sampling bottle with sample water three times.

2. The uncapped bottle was faced down and perpendicular to the creek at arm's length. Submerged it straight down into the water, rotated it to point upstream, and then tilted it slightly up until it fills with water.

3. Tap the sides of the submerged bottle to dislodge any air bubbles clinging to the inside. Replaced cap, while the bottle is still submerged.

4. Retrieved bottle and examined it carefully to make sure that no air bubbles were trapped inside.

The number of the examined fishes was 5(five). All fishes were approximately weighted 150grn.

3.5 Sample Preparation:

3.5.1 Mucus: A 1cm x 1 cm area on the fish body was selected and the mucus present in that particular area was scrapped off and exactly 1.0 gm was weighted out,then suspension of mucus was made in 10 ml of sterile physiological saline (0.9% NaCl).

3.5.2. Intestine: Then the fish were dissected longitudinally from the ventral side. From the exposed peritoneal cavity middle part of the intestine was removed and exactly 1.0 gm was weighted out and pasted well in a sterile mortar pestle with 1 ml of Physiological saline. The mixture was then suspended in 9 ml of sterile physiological saline to make a final volume of 10 ml.

3.5.3. Water: No special treatment was done with water sample. Collected water sample was considered as concentrated inoculum and later serial dilutions were prepared from it.

3.5.4. Fish Feeds: No special treatment was done with water sample. Exactly 1.0 gm was weighted out and pasted well in a sterile mortar pestle with 1 ml of Physiological saline. The mixture was then suspended in 9 ml of sterile physiological saline to make a final volume of 10 ml.

3.6 Bacteriological Studies:

3.6.1. Isolation Of Bacteria: For the isolation of bacteria, the streak and spread plate methods were followed. Instant MacConkey((Biolife, Italiana S.r.1), Nutrient (DIFCO Manual, 1984), SS(Biolife, Italiana S.r.1) and TCBS(Biolife,italialia S.r.1) agar media were used for the isolation purpose . All the media were prepared according to the instructions given in the label of the container.The MacConkey agar was used for the isolation and differentiation of enteric bacteria (DIFCO manual, 1984).This medium also supports the growth of *Staphylococcus* and *Enterococcus.* Nutrient Agar was used for cultivating non fastidious microorganisms.SS agar was used for better growth of *Shigella* and better colony characteristics for *Salmonella.*TCBS agar is highly selective media and it was used for isolating *Vibrio spp.* Different types of bacterial colony grown on MacConkey, SS and TCBS agar were collected and maintained in Nutrient agar slant for further analysis. The Gram negative bacteria were expected to be appeared on these media which are of special interests. Bacterial colonies grown on the Nutrient agar were tested immediately after their growth for morphological and cultural characteristics.

3.7. Preparation Of Culture Media

3.7.1. Nutrient Agar: 1.5 % (Difco Manual, 1984)

Composition (Per liter)

Yeast extract	3.0 g
Peptone(DIFCO)	5.Og
NaCl (BDH chemical UK)	8.Og
Agar	15.Og
Final P^H	7.3 ± 0.1 at 25° C

All the ingredients were suspended in one (1) liter distilled water and heated to boil. The media was then autoclaved at 121°C for 15 minutes. The sterilized media cooled Up to 50-60° C was then poured into previously sterilized petridishes and were allowed to solidify. The Nutrient agar was used for cultivating non-fastidious microorganisms.

3.7.2. Macconkey Agar (Biolife, Italians S.R.1):

Composition (per liter)

Peptone Bacteriological	17.0 g
Peptone complex	3.0 g
Lactose	10.0 g
Bile Salt N 3	1.5 g
NaCI	5.0 g
Neutral red	0.03 g
Crystal violet	0.001 g
Agar	13.50 g
Final P^H	7.1±0.2 at 25° C

50.0 grams of MacConkey agar powder (instant) was dissolved in 1000 ml of cold deionize distilled water. Then allowed to soak for 10 minutes, swirl to mix and heated to boil. Then the media was autoclaved at 121°C (1, 06 kg/cm^2) for 15 minutes, cooled up to 50-60 ° C and mixed well before pouring into previously sterilized petridishes. Media was then poured into petriplates and allowed to solidify. Prior to inoculation the surface of the agar was dried by partially exposed them at 37°C in clean bench. The MacConkey agar was used for the isolation and differentiation enteric bacteria (DIEGO Manual, 1984) .This medium also supports the growth of *Staphylococcus* and *Enterococcus.*

3.7.3 Salmonela Shigella Agar (Biolife, Italiana S.R.I):

Composition (per litre)

Beef extract	5.0 g
Peptone or trypose	5.0 g
Lactose	10.0 g
Bile Salt	8.5 g
Sodium citrate	8.5 g
Sodium thiosulphate	8.5 g
Ferric citrate	1.0 g
Neutral red	0.025 g
Brilliant green	0.33 g
Agar	13.5
Final P^H	7.3 ± 0.2 at 25° C

56.70 g of SS agar powder (ready made) was suspended in 1000 ml of demonized water. The suspension was brought to boiling with frequent agitation and allowed to simmer gently to dissolve the agar. Media was then cooled to 50°C and poured into sterilized petridishes. The media was not autoclaved. SS agar was used for the better growth of *Shigella* and *Salmonella.*

3.7.4. Thiosulphate Citrate Bile Salt Sucrose

Composition (per liter)

Tryptone	10.g
Yeast extracts	5.0g
Sodium Thiosulphate	10.0g
Sodium citrate	10.0g
NaCl	10.0g
Ox Bile Bios	8.0g
Sucrose	20.0g
Ferric citrate	1.0g
Bromothymol blue	0.04g
Thymol blue	0.04g
Agar	16.0g
Final P"	8.4±0.2 at 25°C

90.0 g of TCBS agar powder (instant) was added to 1000 ml of deionized water. The suspension was allowed to soak for 10 minutes. Swirl to mix and then it was brought to boiling. The media was then cooled to 50°C and poured into sterile petridishes. The media was not autoclaved. The TCBS agar is a highly selective media for isolating *Vibrio spp*.

3.7.5. Preparation Of Diluent (Physiologicsalline):

Physiological saline was used as sample diluent. This was prepared by adding 0.9 gm o NaCl in 100 ml distilled water. With this concentration of NaCl the amount of physiological saline was prepared as per requirement. Aliquots of 9ml of the saline were distributed in test tubes and autoclaved at 121°C for 15 minutes.

3.8. Isolation Procedure:

3.8.1. Preparation of bacterial dilution: Dilution was made from the concentrated bacterial suspension by tenfold serial dilution method (Pciczer ct. al. 1993). From concentrated bacterial suspension 1ml was transferred to a test tube containing 9 ml of physiological saline. Then the solution was homogenized with vortex. Then 1 ml of suspension from this tube was transferred to the next tube containing 9 ml saline and so on.

3.8.2. Inoculation: From the desired dilution 20 µl amount of sample suspension was transferred onto previously prepared Nutrient, MacConkey, SS and TCBS agar surface in petridishes by micropipette and spreaded with sterile glass spreaders. All these steps were done inside laminar air flow hood to maintain aseptic conditions.

3.8.3. Incubation: All the inoculated plates were incubated at 37°C in an incubator for 24 to 48 hours. All the petridishes were kept in incubator under 37°C over night before inoculating to avoid contamination.

3.8.4. Total Viable Count: After 24 -48 hours of incubation, bacterial colonies were appeared on the agar surface. The colonies appearing on the plates were counted by the following formula.

CFU/g = Number of colonies appeared x Amount of sample applied on Petridish x Dilution Factor

3.8.5. Isolation Of Bacterial Colonies:

After the bacterial colonies were appeared on the agar surfaces clear, discrete and isolated colonies with different color, margin surface elevation and shape were selected for pure culture. The selected colonies were marked and each colony was picked up separately by an inoculum loop and inoculated in nutrient agar slant.

3.8.6 Preparation Of Agar Slant For Storage Of The Bacterial Isolates:

Nutrient agar media of the following composition was used to maintain the individual bacterial culture (strains).

Nutrient Agar 1.5 %(DIFCO Manual, 1984)

Composition (Per litre)

Yeast extract	3.0 g
Peptone (DIFCO)	5.0 g
NaCI(BDI-I chemical UK) Agar	8.0g
Final P^H	15.0 g

All the ingredients were suspended in distilled water and heated to boil. 5 ml media was dispensed in each test tube and plugged with cotton. Then it was autoclaved at 121°C for 15 minutes. After autoclaving test tubes were kept in sloping position of 30°- 45° A for solidification.

3.9. Preservation And Maintenance:

From the culture plates, the discrete and isolated single colony of each type was transferred to agar slants and incubated. After 24 hours bacterial growths were observed. The agar slants were kept in the refrigerator at 4°C as stock culture for future studies. For proper maintenance, these slant culture were sub cultured in every 15 days.

3.10. Coding Of Isolates:

Bacterial isolates from Nutrient,MacConky, SS and TCBS agar were coded according to the names of the media , fish site and sample number . The coding was done as follows

Every sample had been taken for twice i.e. sample a and sample b

NIS_{1a} = Nutrient agar, Intestine, Sample No.1a.

NIS_{1b} = Nutrient agar, Intestine, Sample No.lb.

NlS_2 = Nutrient agar, Intestine, Sample No.2.

NIS_3 = Nutrient agar, Intestine, Sample No.3.

NIS_4 = Nutrient agar, Intestine, Sample No.4.

NIS_5 = Nutrient agar, Intestine, Sample No.5.

Sample coding was assigned by following the above way for different working media

MIS= MacConky Agar, Intestine, Sample No

SIS = SS Agar, Intestine, Sample No

TIS= TCBS Agar, Intestine, Sample No

NMS= Nutrient agar, Mucus, SampleNo

MMS= MacConky Agar, Mucus, Sample No

SMS = SS Agar, Mucus, Sample No

TMS = TCBS Agar, Mucus, Sample No

Three water samples were collected from three different locations of the same rearing pond (Sample number = 3, Duplication of cach sample as sample a and sample b)

NWS_{1a}= Nutrient agar, Water, Sample No. 1a.

NWS_{1b} = Nutrient agar, Water, Sample No. lb.

Sample coding was assigned by following the above way for different working media

MWS= MacConky Agar, Water, Sample No.

SWS = SS Agar, Water, Sample No.

TWS = TCBS Agar, Water, Sample No.

Two Fish Feed samples were collected from three different sources (Sample number =2, Duplication of each sample as sample a and sample b)

$NFFS_{1a}$= Nutrient agar, Fish Feed-1, Sample No.1a

$NFFS_{1b}$ = Nutrient agar, Fish Feed-1, Sample No.1 b.

Sample coding was assigned by following the above way for different working media

$MFFS_{1a}$= MacConky agar, Fish Feed-1, Sample No.1a

$SFFS_{1a}$= SS Agar, Fish Feed-1, Sample No.1a

$TFFS_{1a}$ = TCBS Agar, Fish Feed-1, Sample No.1a

$NFFS_{2a}$= Nutrient agar, Fish Feed-2, Sample No.2a

$NFFS_{2b}$ = Nutrient agar, Fish Feed-2, Sample No.2b.

Sample coding was assigned by following the above way for different working media

$\overline{MFFS_{2a}}$= MacConky agar, Fish Feed-2, Sample No.2a

$SFFS_{2a}$= SS Agar, Fish Feed-2, Sample No.2a

$TFFS_{2a}$ = TCBS Agar, Fish Feed-2, Sample No.2a

The code numbers were maintained and followed till the end of the study.

3.11. Characterization Of The Isolates:

To identify the isolated strains cultural, morphological and biochemical characteristics were studied carefully according to the methods described by Buchanan and Gibbons (1974).

3.11.1. Cultural Characteristics:

The bacterial colonies grown on respective media were studied for their color, colony shape, size, margin, surface, elevation, and growth on Agar or in Midia.

Surface colonies on the solid media:

Whole colony/Colony shape: Punctiform, Circular, Rhizoid, Irregular.

Rhizoid

Irregular

Circular

Punctiform (pin-point): Less than 1 mm in diameter.

Size: Have to record diameter in mm.

Color: Yellow, Pink, Purple, Brown Black, Creamy,Gray, Colorless, etc.

Elevation: Flat, Raised, Convex, Umbonate

Surface: Smooth, Rough, Glistening, Dry, And Powdery.

Margin/Edge: Entire, Undulate, Lobate.

Growth: On Surface, In Media.

3.12. Morphological Characteristics:

3.12.1. Staining Of Bacteria:

Cleaned and grease free microscopic glass slides were used for this purpose. The slides were washed and dried. After drying, the slides were rubbed by cotton soaked with 95% ethanol

3.12.2. Staining Technique:

Preparation of smear on slides:

a) A clean slide was taken and a small drop of distilled water was placed on the middle of the slide with a loop.

b) A very small portion of freshly prepared bacterial culture from stock was transferred on the slide by inoculating loop and a very thin film was prepared by spreading it uniformly.

c) The film was fixed by passing the slide quickly over the gentle flame of spirit lamp for 1-2 times.

Procedure: A drop of 1% Methylene blue was placed on fixed film and allowed to stand for 30 minutes .Then it was washed with tap water and soaked the excess water with tissue paper.

3.13. Cell Morphology: The stained slides were viewed by oil

immersion objective (100x10).The shape, sizes of the individual bacterial cells were observed. The arrangement of the cells were also studied and recorded.

3.13.1. Gram Staining:

Ingredients:

1. 0.5% crystal violet solution.
2. Lugol's Iodine solution as mordant (l% iodine in 2% potassium iodide).
3. 95% ethanol (Alcohol).
4. Safranin solution. (O. I% safranin in 2% acetic acid).

Gram stain procedure is as follows:

1. A slide with a bacterial smear was placed on a staining rack.
2. The slide was stained with crystal violet for 1-2 min.
3. Pour off the stain.
4. The slide was flooded with Gram's iodine for 1-2 min.
5. Pour off the iodine.
6. Decolorized the slide by washing briefly with alcohol (10 seconds).
7. The slide was washed thoroughly with water to remove the alcohol. It is recommended not to delay with this step.
8. The slide was then flooded with safranin counter stain for 2 min.
9. Wash with water.
10. Blot excess water and dry in hand over Bunsen flame.
11. The slides were then examined under bright light field microscope (Olympus) at a magnification of 10x100 by Oil immersion object.

3.14. Biochemical Characteristics: The isolates were characterized for their biochemical properties. The following biochemical tests were done for the identification of the bacterial strains which had been grown on MacConkey, SS and TCBS agar.

1. KIA
2. MIU.
3.Catalae
4.Oxidae

.

2.14.1 Kligler's Iron Agar (KIA) Oxoid, Uk

Composition (per litre)

Meat infusion broth	1.0 Liter
Peptone	5.0
Protcose peptone	5.0 g
Sodium chloride	5.0
Lactose	10.0 g
Glucose	1.0 g
Ferrous sulphate	0.2 g
Sodium thiosulphate,	0.3 g
Ferric citrate	1.0 g
Agar	12.Og

Preparation:

The desired amount of KIA powder (instant) was suspended in the distilled water. Heating dissolved the agar in the suspension. Then 6-7ml amounts of media were distributed in the test tubes. The tubes were then sterilized by autoclaving under 1.06 kg/cm^2 pressures at 121°C for 15

minutes. The media was then cooled and set with a slant of 2.5 cm and a butt of 2.5 cm deep.

3.14.2 Sterility Test:

The tubes were incubated at 37°C for 18-24 hours and examined for contamination of sterility. If contamination occurred, there would be the growth of unwanted bacterial colonies. Contaminated tubes were discarded and further investigation was done with only sterile media.

3.14.3. Motility Indole Urea (MIU):

Composition (per litre)

Peptone (rich in tryptophane)	30g
KH_2PO_4 (S.D.Fine CHEM .Ltd)	1.Og
Agar	5.Og
Phenol red (Alcoholic solution) (E.Merck,India)	2.0 ml
Distilled water	1 liter

Alcoholic solution of phenol red was prepared by dissolving 0.25g of phenol red in 50m1 ethyl alcohol and adding 50 ml of distilled water.

Preparation: The ingredients were added to distilled water, boiled to dissolve and filtered through paper. 90 ml quantities were distributed in screw cap tubes and sterilized by autoclaving under 1.06 kg/cm2 pressures at 121 'C for 15 minutes.

A 20% urea solution was prepared. Urea solution(SIGMA,UK) was sterilized by filtration through microbiological filter.10ml of 20% sterile urea solution was added to 90 ml of culture medium that has been melted and cooled to 50°C.Then the media was distributed in presterile test tubes

by 5 ml in each and allowed to solidify in an upright position.

Iodole paper preparation: Iodole reagent was prepared according to the following composition.

Para dimethyl amino benzaldehyde (Merck,lndia)	5.0 g
Isoamyl alcohol (Merck, India)	75.0ml
Hydrochloric acid (concentrated) (Merck , India)	25.0 ml

Benzaldehyde was dissolved in isoamyl alcohol and then hydrochloric acid was added. Whatman double filter paper was cut into small strips of 5 x 0.5cm size. Filter paper strips were soaked with indole reagent and dried at 37°C over night.

3.14.4. Performance Of KIA And MIA Tests:

Both the tests were performed according to the methods described in the manual (Manual for laboratory investigation of acute enteric infections, WI-10).Using a straight wire, an inoculums from the top of the selected colony avoiding the underlying agar was taken. Smooth colonies were selected. Then inoculum was inoculated in the KIA first. The butt was stabbed and then the slant was streaked with a zig-zag conformation. The tip of the wire was touched to the point of the stab in the Kathleen stabbed the MIU without retouching the colony or flaming the wire. A strip of filter paper that has been saturated with Indole reagent and dried, was placed on the inside top of the MIU tube and the tube was kept in place with cotton plug. 'Then both types of tubes were placed for overnight incubation at 37°C. Fermentation of glucose was indicated by the production of acid (yellow butt) or alkali (red) and gas on KIA. Gas production from glucose was indicated by bubbles or creaks throughout the medium. Large amount

of gas indicated by the disruption of the media. Lactose fermentation simultaneously with glucose was indicated by yellow colorization of both butt and slant. If lactose is not fermented then the butt will be yellow and slant will be red (alkaline).

3.14.5. Motility:

Motility was indicated by spreading growth from the area of the stab line. Observations for clarify of the medium in comparison with non motile cultures are done by holding an uninoculated tube of MIU medium next to the tube with the test strain .

3.14.6. Urease Activity:

Unease activity was indicated by a red color in the MIU medium. The red color appears when urea was hydrolyzed causing an alkaline P^H

The presence of iodole was read by two ways.

1. The paper strip impregnated with Kovac's reagent attached to the top of the tube turned red if indole was produced.

2.If test was questionable or negative, 1 ml of iodole reagent was added to the surface of the MIU medium. The presence of iodole turned the surface layer red within 10 minutes.

3.14.7. Catalase Test: Catalase test was done to detect the presence of the enzyme catalase. Catalase enzyme is found in most bacteria. It catalyses the breakdown of hydrogen peroxide (H_2O_2) with the release of free Oxygen.

Method

I. Dipcapillary tube was dipped into 30 %H_2O_2.

2. The colony was touched with the tube.

3. Formation of bubble indicating positive reaction.

3.14.8. Oxidase Test: Oxidase Test was done to determine the presence of oxidase enzymes. The reagent (impregnated into strips of double filter paper) contains tetrametliyl-p-plienylenediamine which serves as an alternate substrate for the cytochrome oxidase reaction. In the reduced state the reagent was colourless but when oxidised it became purple.

Method: A piece of the oxidase test paper was touched onto an area of heavy bacterial growth with the help of a forcep. The results were interpreted as follows,

Colour change to purple within -

10 seconds = positive

10 - 60 seconds = delayed positive

>60 seconds = negative

3.15. Water Parameters:

1. DO
2. BOD
3. P^H
4. Temperature

3.15.1. Dissolved Oxygen (Do):

Test Equipment

1. LaMotte Dissolved Oxygen Test Kit (Code 5856)
2. Gloves and goggles
3. Towel
4. Waste container
5. Small brown paper bag

Procedure:
Collection &Treatment of the Water Sample

Water samples were collected with special care and followed the following steps.

1. Putted on gloves and goggles. To avoid contamination, thoroughly rinse the water sampling bottle with sample water three times.

2. The uncapped bottle was faced down and perpendicular to the creek at arm's length. Submerged it straight down into the water, rotated it to point upstream, and then tilted it slightly up until it fills with water.

3. Tap the sides of the submerged bottle to dislodge any air bubbles clinging to the inside. Replaced cap, while the bottle is still submerged.

4. Retrieved bottle and examined it carefully to make sure that no air bubbles were trapped inside.

5.Repeated the step from 1-4 with a second sample bottle, which was used for the 5-day biochemical oxygen demand (BOD) test, for which a dissolved oxygen measurement was also needed.

6. Dried off the second bottle with the towel and placed it into the brown paper bag to shield it from light and continued the procedure with the first sample bottle. The towel was used to catch any spills from subsequent steps.

When a satisfactory sample had been collected, proceed immediately with steps 5 and 6 to "fix" the sample. We had to careful not to introduce air into the sample while adding the reagents in steps 5 & 6. Simply drop the reagents into sample. Capped carefully, and mixed gently.

7.8 drops of manganous sulfate solution and 8 drops of alkaline potassium iodide azide were added .The dropper was kept perpendicular and hold approximately one inch above the sample bottle so that drop size would be consistent. The sample bottle was capped and mixed by inverting several times. A precipitate was form. Allowed the precipitate to settle below the shoulder of the bottle before proceeding (1-5 minutes).

8. 8 drops of sulfuric acid was used .Capped and gently shaked until the reagent and the precipitate had been dissolved. A clear-yellow to brown-orange color was developed, depending on the oxygen content of the sample.

9. Filled the graduated cylinder to 20 ml line with "fixed" sample.

10.Transfered to titration tube and inserted the direct reading titrator into the hole at the mouth of the bottle containing sodium thiosulfate, 0.025N, turned the bottle upside-down and pulled the titrator plunger gently, filling the graduated tube until the tip of the plunger lines up with the zero mark. The titrator plunger had a tendency to stick at the bottom or wherever it had been stored.

11. Inserted the titrator into the center hole of the titration tube cap. While gently shaking the tube, slowly pressed the plunger to titrate until the yellow-brown color is reduced to a very faint yellow.

12. Removed the titrator and cap. 8 drops of starch indicator solution was added to the titration tube. Sample would turn blue.

13. Replaced the cap and titrator. Continued titrating until the blue color just disappeared. When the blue became pale, pressed the titrator plunger very slowly, allowed only 1/2 drop at a time. Always swirl thoroughly after each drop. Read the test result where the plunger tip meets the scale. Record as ppm dissolved oxygen.

Each minor division on the titrator scale equals 0.2 ppm.

If the plunger tip reaches tile bottom line on the titrator scale (10 ppm) before the end point color change occurs, refilled the titrator a small amount and continued the titration.

3.15.2. Biological Oxygen Demand (Bod)

Test Procedure:

The BOD test took 5 days to complete and was performed using a dissolved oxygen test kit (from LaMotte).Record the dissolved oxygen level (in ppm) on the first day using the method .Then place the water sample in an incubator at 20°C for 5 days or wrap the water sample bottle in aluminum foil or black electrical tape and store in a dark place at room temperature (20 C or 68 F). On day 5, took another dissolved oxygen reading (in ppm) using the dissolved oxygen test kit. The BOD level was determined by subtracting the Day 5 reading from the Day 1 reading. Record the final BOD result in ppm.

P ^{H} and Temperature:

The P^{H} was measured by a p^{H} meter. At first, the P^{H} meter was calibrated by three calibrators. JENWAY 3010 P^{H} meter, (German) has three calibrators of different values. Those are P^{H} 4.0(acidic), P^{H} 7.0(neutral) and P^{H} 10.0(alkaline).Then the machine was ready for measuring p^{H} of the water sample. Temperature of the water samples were measured by a special thermometer.

3.15.3. Proximate Composition Of Fish Feed-1 And Fish Feed-2:

Proximate composition of fish feed samples (FFS-1 and FFS-2) was analyzed by the standard methods of AOAC [4].

Chepter-4:

- Results

4. Results

Table-1: Total bacterial count of mucus of Open Water Tilapia (*Oreochromis niloticus*) grown on Nutrient and MacConky agar

Sample No	Nutrient Agar (cfu/g)	Average (cfu/g)	MacConkey Agar(cfu/g)	Average (cfu/g)
Fish 1	9.95×10^5	9.9×10^5	1.17×10^5	1.48×10^5
Fish 2	10.01×10^5		1.87×10^5	
Fish 3	9.48×10^5		1.96×10^5	
Fish 4	10.15×10^5		1.10×10^5	
Fish 5	9.96×10^5		1.31×10^5	

The total viable bacterial count in the mucus of Open Water Tilapia fish on Nutrient and MacConkey agar were presented in table no 1. As shown in the table 1 on Nutrient agar the average bacterial counts(cfu/g) were 9.9×10^5 where on MacConkey agar the bacterial counts were 1.48×10^5 (cfu/g).

Table-2: Total bacterial count of the intestine of Open Water Tilapia (*Oreochromis niloticus*) grown on Nutrient and MacConkey agar

Fish Sample	NutrientAgar (cfu/g)	Average (cfu/g)	MacConkeyAgar (cfu/g)	Average (cfu/g)
Fish 1	1.80×10^6	1.85×10^9	1.40×10^6	1.73×10^8
Fish 2	1.95×10^6		1.80×10^6	
Fish 3	1.90×10^6		1.95×10^6	
Fish 4	1.75×10^6		1.75×10^6	
Fish 5	1.85×10^6		1.75×10^6	

The total viable bacterial count in the intestine of Open Water Tilapia fish on Nutrient and MacConkey agar were presented in table no 1. As shown in the table 1 on Nutrient agar the average bacterial counts (cfu/g) were 1.85×10^9 where on MacConkey agar the bacterial counts were 1.73×10^8 (cfu/g).

Table-3: Total bacterial count of mucus of Cultured Tilapia (*Oreochromis niloticus*) grown on Nutrient and MacConkey agar

Fish Sample	NutrientAgar (cfu/g)	Average (cfu/g)	MacConkey Agar (cfu/g)	Average (cfu/g)
Fish 1	1.32×10^5	1.29×10^5	6.87×10^4	7.03×10^4
Fish 2	1.23×10^5		7.33×10^4	
Fish 3	1.47×10^5		7.08×10^4	
Fish 4	1.20×10^5		7.25×10^4	
Fish 5	1.25×10^5		6.66×10^4	

The total viable bacterial count in the mucus of Cultured Tilapia fish on Nutrient and MacConkey agar were presented in table no 3. From the table it was observed that on Nutrient agar the average bacterial counts (cfu/g) for mucus were 1.29×10^5 where on MacConkey agar the bacterial counts were 7.03×10^4 (cfu/g).

Table-4: Total bacterial count of the intestine of Cultured Tilapia (*Oreochromis niloticus*) grown on Nutrient and MacConkey agar media

Fish Sample	NutrientAgar (cfu/g)	Average (cfu/g)	MacConkeyAgar (cfu/g)	Average (cfu/g)
Fish 1	4.85×10^6	4.96×10^6	3.45×10^6	3.90×10^6
Fish 2	4.20×10^6		3.36×10^6	
Fish 3	5.66×10^6		3.80×10^6	
Fish 4	5.29×10^6		4.28×10^6	
Fish 5	4.82×10^6		4.62×10^6	

The total viable bacterial count in the intestine of Cultured Tilapia fish on Nutrient and MacConkey agar is presented in table no 4. From the table it was observed that on Nutrient agar the average bacterial counts (cfu/g) were 4.96×10^6 where on MacConkey agar the bacterial counts were 3.90×10^6 (cfu/g).

Table-5: Comparison between Open Water Tilapia and Cultured Tilapia, in respect to the average total bacterial count of the intestine and mucus of Tilapia (*Oreochromis niloticus*)grown on Nutrient and MacConkey agar media

Fish type	Intestine (cfu/g)		Mucus (cfu/g)	
	Nutrient	MacConkey	Nutrient	MacConkey
Cultured	4.96×10^6	3.90×10^6	1.29×10^5	7.03×10^4
Open Water	1.85×10^9	1.73×10^8	9.9×10^5	1.48×10^5

From the table 5, it was observed that the Culture Tilapia fishes carried lower bacterial content than Open Water Tilapia in both intestine and mucus on Nutrient and MacConkey agar media .On Nutrient agar the average bacterial counts (cfu/g) were 4.96×10^6 where on MacConkey agar the bacterial counts were 3.90×10^6 (cfu/g). On Nutrient agar the average bacterial counts (cfu/g) for mucus were1.29×10^5 where on MacConkey agar the bacterial counts were 7.03×10^4 (cfu/g).

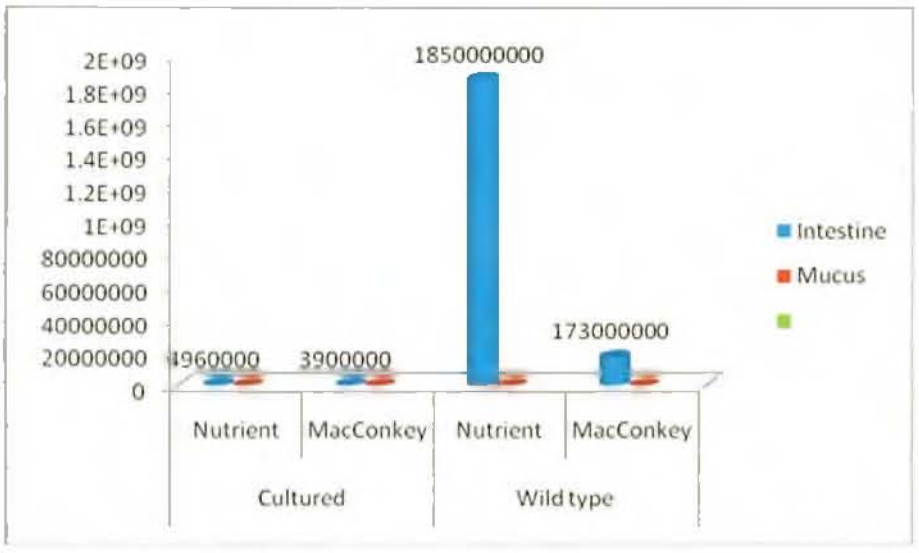


Figure 1: Comparison between Open Water Tilapia and Cultured Tilapia, in respect to the average total bacterial count.

Table-6: Cultural characteristics of bacterial cells isolated from mucus of Open Water Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS

Strain	Colony shape	Color	Margin	elevation	Surface	Growth on Agar
NMS_{1a}	Circular	Purple	Entire	Convex	Smooth	Surface
NMS_{1b}	x	x	x	x	x	x
NMS_{2a}	Circular	Pale yellow	Undulate	Raised	Smooth	Surface
NMS_{2b}	Circular	Creamy	Entire	Convex	Smooth	Surface
NMS_{3a}	Circular	White	Entire	Convex	Smooth	Surface
NMS_{3b}	x	x	x	x	x	x
NMS_{4a}	Circular	yellow	Entire	Convex	Smooth	Surface
NMS_{4b}	Circular	Creamy	Entire	Flat	Smooth	Surface
NMS_{5a}	Circular	Gray	Entire	Convex	Glistening	Surface
NMS_{5b}	Circular	Light White	`Entire	Flat	Smooth	Surface
MMS_{1a}	Circular	Creamy	Entire	Raised	Smooth	Surface
MMS_{1b}	Circular	Creamy	Entire	Convex	Smooth	Surface
MMS_{2a}	Circular	Purple	Entire	Convex	Smooth	Surface
MMS_{2b}	Circular	Pale yellow	Entire	Convex	Smooth	Surface
MMS_{3a}	Circular	Creamy	Undulate	Flat	Smooth	Surface
MMS_{3b}	Circular	Brown	Entire	Flat	Smooth	Surface
MMS_{4a}	Circular	Pink	Irregular	Flat	Smooth	Surface
MMS_{4b}	Circular	Pink	Entire	Convex	Smooth	Surface
MMS_{4c}	Circular	Pale yellow	Undulate	Raised	Smooth	Surface
MMS_{5a}	x	x	x	x	x	x
MMS_{5b}	Circular	Colorless	Entire	Convex	Smooth	Surface
SMS_{1a}	Circular	Pink	Entire	Flat	Smooth	Surface
SMS_{1b}	Circular	Purple	Entire	Convex	Rough	Surface
SMS_{2a}	Circular	Colorless	Entire	Convex	Smooth	Surface
SMS_{2b}	Circular	Creamy	Entire	Flat	Glistening	Surface
SMS_{3a}	x	x	x	x	x	x
SMS_{3b}	Circular	Creamy	Entire	Convex	Smooth	Surface
SMS_{4a}	Circular	Colorless	Entire	Convex	Smooth	Surface
SMS_{4b}	Circular	Pink	Entire	Convex	Glistening	Surface
SMS_{4c}	Circular	Colorless	Entire	Convex	Smooth	Surface
SMS_{5a}	Circular	Pink	Entire	Convex	Smooth	Surface
SMS_{5b}	Circular	Creamy	Entire	Convex	Smooth	Surface
$TMS_{1(a+b)}$	x	x	x	x	x	x
$TMS_{2(a+b)}$	x	x	x	x	x	x
$TMS_{3(a+b)}$	Circular	yellow	Entire	Flat	Glistening	Surface
TMS_{4a}	Circular	yellow	Entire	Flat	Glistening	Surface
TMS_{4b}	x	x	x	x	x	x
TMS_{5a}	Circular	yellow	Entire	Flat	Glistening	Surface
TMS_{5b}	x	x	x	x	x	x

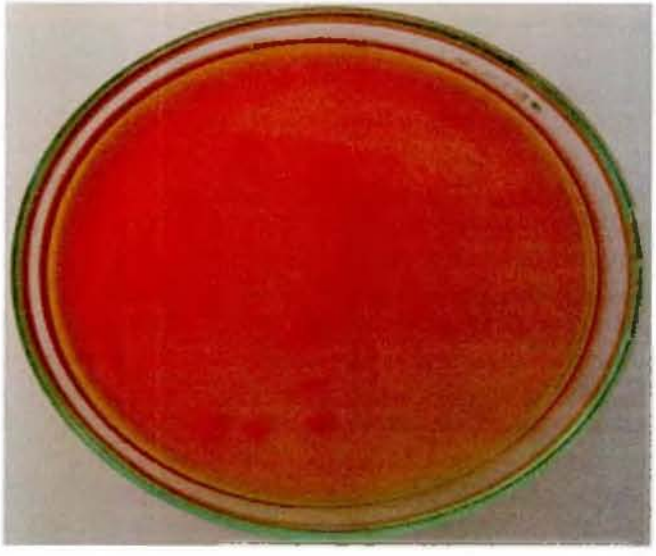
x = No growth, NMS= Nutrient Agar Mucus Sample, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample.

According to table no. 6 from the mucus of Open Water Tilapia -

Total strains were 42 and growths were not found in 10 strains. 32 circular colonies were shown. Colonies of different colors were chosen such as creamy, yellow, pale yellow, light white, pink, white, purple, brown, colorless and gray. 28 colonies showed entire margin, 1 irregular margin and 3 colonies showed undulate margin. 18 colonies showed convex surface elevation, 11 colonies showed flat surface elevation, and 3 colonies showed raised elevation. 24 colonies showed smooth surface, 1 showed rough surface and 7colonies showed glistening surface .All growth were observed on agar surface.

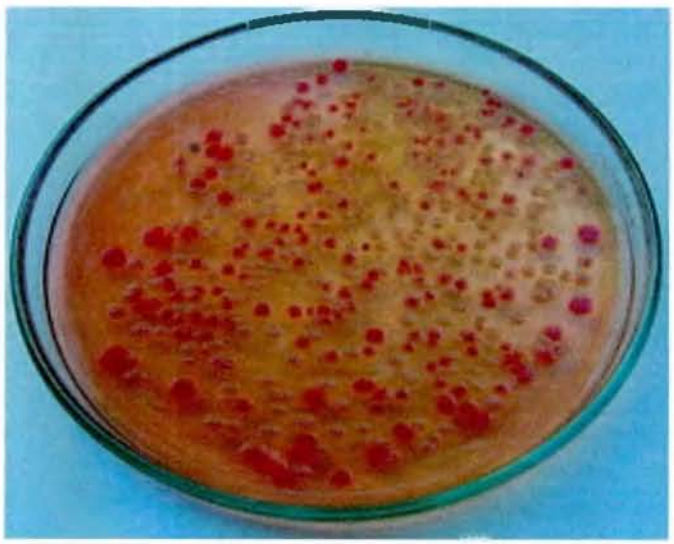
Picture-1.a:
Cultural characteristics from mucus of Open Water (Lake) Tilapia (*Oreochromis niloticus*) Grown on Nutrient Agar and MacConkey Agar media.

Picture-1.a.1: Nutrient Agar.

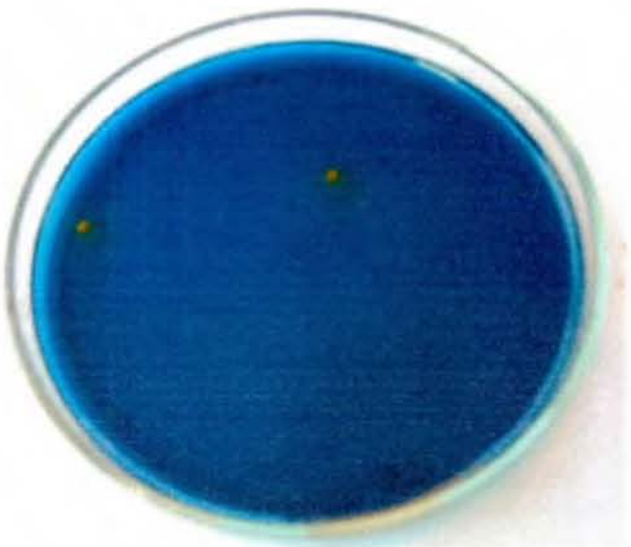


Picture-1.a.2: MacConkey Agar.

Picture-1.b:
Cultural characteristics from mucus of Open Water (Lake) Tilapia (*Oreochromis niloticus*) Grown on SS Agar and TCBS Agar media.



Picture-1.3: SS Agar.



Picture-1.4: TCBS Agar.

Table-7: Morphological characteristics of bacterial cells isolated from mucus of Open Water Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
NMS_{1a}	-	Rod	Arranged in single cell
NMS_{1b}	x	x	x
NMS_{2a}	-	Short Rod	Arranged in small chain
NMS_{2b}	-	Rod	Arranged in long chain
NMS_{3a}	-	Rod	Arranged in small chain
NMS_{3b}	x	x	x
NMS_{4a}	-	Rod	Arranged in single and pair
NMS_{4b}	-	Rod	Arranged in single and pair
NMS_{5a}	-	Rod	Arranged in single and pair
NMS_{5b}	-	Rod	Arranged in single cell
MMS_{1a}	-	Rod	Arranged in single and pair
MMS_{1b}	-	Short rod	Arranged in small chain
MMS_{2a}	-	Short rod	Arranged in small chain
MMS_{2b}	-	Rod	Arranged in single cell
MMS_{3a}	-	Rod	Arranged in long chain
MMS_{3b}	-	Rod	Arranged in long chain
MMS_{4a}	-	Rod	Arranged in single and pair
MMS_{4b}	-	Rod	Arranged in long chain
MMS_{4c}	-	Rod	Arranged in small chain
MMS_{5a}	x	x	x
MMS_{5b}	-	Rod	Arranged in single and pair
SMS_{1a}	-	Rod	Arranged in single and pair
SMS_{1b}	-	Rod	Arranged in single and pair
SMS_{2a}	-	Rod	Arranged in small chain
SMS_{2b}	-	ShortRod	Arranged in small chain
SMS_{3a}	x	x	x
SMS_{3b}	-	Rod	Arranged in long chain
SMS_{4a}	-	Rod	Arranged in single and pair
SMS_{4b}	-	Rod	Arranged in single and pair
SMS_{4c}	-	Rod	Arranged in single and pair
SMS_{5a}	-	Rod	Arranged in single and pair
SMS_{5b}	-	Rod	Arranged in single and pair
$TMS_{1(a+b)}$	x	x	x
$TMS_{2(a+b)}$	x	x	x
$TMS_{3(a+b)}$	-	Curved Rod	Arranged in single and pair
TMS_{4a}	-	Curved Rod	Arranged in single and pair
TMS_{4b}	x	x	x
TMS_{5a}	-	Curved Rod	Arranged in single and pair
TMS_{5b}	x	x	x

x = No growth, NMS= Nutrient Agar Mucus Sample, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample.

According to table no 7 from the mucus of Open Water Tilapia -

Total strains were 42. But growths were observed in 32 strains and no growth found in 10 strains. All of them, 24 isolates were rod and gram negative and 4 were short rod and gram negative.4 were curved rod and grain negative. Among them 18 were arranged in single and pair, 6 were in small chain, 5 were long chain and 3 were single cell.

Table-8: Biochemical characteristics of gram negative bacterial cells isolated from mucus of Open Water Tilapia (*Oreochromis niloticus*) grown on different culture media (MacConkey, SS and TCBS)

Stain	Gram reaction	Slant	Butt	Gas	Motility	Indole	Urease	Catalase	Oxidase	Isolates
MMS_{1a}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MMS_{1b}	-	K	A	-	+	+	-	+	+	*Pseudomonas*
MMS_{2a}	-	A	A	+	-	+	+	+	-	*Klebsiella*
MMS_{2b}	-	K	A	-	+	+	-	+	+	*Plesiomonas*
MMS3a	-	A	A	+	+	-	-	+	-	*E.coli*
MMS_{3b}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MMS_{4a}	-	A	A	+	+	-	-	+	-	*Enterobacter*
MMS_{4b}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MMS_{4c}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MMS_{5a}	x	x	x	x	x	x	x	x	x	x
MMS_{5b}	-	K	A	-	+	+	-	+	+	*Pseudomonas*
SMS_{1a}	-	K	A	+	+	-	-	+	-	*Salmonella*
SMS_{1b}	-	K	A	-	-	+	-	+	-	Unidentified
SMS_{2a}	-	K	A	-	-	-	-	+	-	*Shigella*
SMS_{2b}	-	K	A	-	-	-	-	+	-	*Shigella*
SMS_{3a}	x	x	x	x	x	x	x	x	x	x
SMS_{3b}	-	A	A	+	+	-	-	+	-	Unidentified
SMS_{4a}	-	K	A	+	+	-	-	+	-	*Salmonella*
SMS_{4b}	-	A	A	-	-	-	-	+	-	Unidentified
SMS_{4c}	-	K	A	+	+	-	-	+	-	*Salmonella*
SMS_{5a}	-	K	A	-	-	-	-	+	+	Unidentified
SMS_{5b}	-	K	A	+	+	-	-	+	-	*Salmonella*
TMS_1(a+b	x	x	x	x	x	x	x	x	x	x
TMS_1(a+b)	x	x	x	x	x	x	x	x	x	x
TMS_3(a+b)	-	K	A	-	+	+	-	+	+	*Vibrio*
TMS_{4a}	-	K	A	-	+	+	-	+	+	*Vibrio*
TMS_{4b}	x	x	x	x	x	x	x	x	x	X
TMS_{5a}	-	K	A	-	+	+	-	+	+	*Vibrio*
TMS_{5b}	x	x	x	x	x	x	x	x	x	x

x = No growth, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample, K = Alkaline (red) reaction, A= Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction

According to table no 8 from the mucus of Open Water Tilapia -

Total strains were 32.Growth was observed in 24 strains and growths were not found in 8 strains. KIA test indicated that out of 24 strains, 5 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 19 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 24 strains, 12 produced gases which were observed by formation of bubbles in the media and 12strains were not produced gas. MIU test indicated that out of 24 strains, 15 strains were motile and 9 were non- motile. Iodole test were positive for 16 strains and negative for 8 strains. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains only 1 showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test:

All the isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 24 gram negative rods, 12 were oxidase positive and 12 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

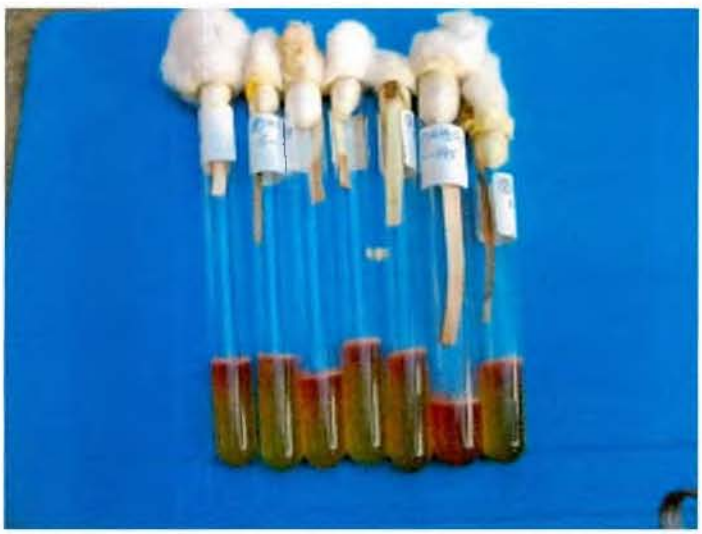
Picture-2:
Biochemical characteristics from mucus of wild Tilapia (*Oreochromis niloticus*) KIA

Picture-2.1:
KIA- Produced Acid from both Glucose & Lactose so that both the butt & slant turned yellow .

Picture-2.2:
KIA -Fermented glucose but not lactose, so that butt turned into yellow and slant remained red.
In some tube, the surface of the media is cracked due to gas production.
In some test tube, due to Hydrogen Sulfide gas production ,it turned black.

Picture-3:
Biochemical characteristics from mucus of open water(lake) Tilapia (*Oreochromis niloticus*) MIU

Picture-3.1:
MIU-Due to Indole test positive, the Indole paper strip turned into red colour in Tube.



Picture-3.2:
MIU-In tube, surface of the media turned reddish due to Urease activity.

Table 9: Cultural characteristics of bacterial cells isolated from the intestine of Open water Tilapia (*Oreochromis niloticus)* grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Colony shape	Color	Margin	Elevation	Surface	Growth on Agar
NIS_{1a}	Circular	Pale yellow	Entire	Convex	Smooth	Surface
NIS_{1b}	Circular	Creamy	Entire	Convex	Smooth	Surface
NIS_{2a}	Circular	Pale yellow	Entire	Convex	Smooth	Surface
NIS_{2b}	Irregular	White	Undulate	Raised	Glistening	Surface
NIS_{3a}	Circular	White	Entire	Convex	Glistening	Surface
NIS_{3b}	Circular	Yellow	Entire	Convex	Glistening	Surface
NIS_{4a}	Circular	Gray	Entire	Convex	Smooth	Surface
NIS_{4b}	Circular	White	Entire	Convex	Smooth	Surface
NIS_{5a}	Circular	Pale yellow	Irregular	Convex	Smooth	Surface
NIS_{5a}	Circular	White	Entire	Raised	Glistening	Surface
MIS_{1a}	Circular	Pale yellow	Entire	Convex	Glistening	Surface
MIS_{1b}	Circular	Pink	Entire	Raised	Glistening	Surface
MIS_{1c}	Circular	Pink	Irregular	Flat	Smooth	Surface
MIS_{2a}	Circular	Creamy	Entire	Convex	Smooth	Surface
MIS_{2b}	Circular	Yellow	Entire	Convex	Smooth	Surface
MIS_{3a}	Circular	Pink	Entire	Convex	Glistening	Surface
MIS_{3b}	Circular	Colorless	Entire	Flat	Glistening	Surface
MIS_{3c}	Circular	Brown	Entire	Flat	Smooth	Surface
MIS_{4a}	Circular	Red	Entire	Flat	Smooth	Surface
MIS_{4b}	Circular	Colorless	Entire	Flat	Smooth	Surface
MIS_{5a}	Circular	Brown	Entire	Flat	Smooth	Surface
MIS_{5b}	Circular	Pink	Irregular	Flat	Smooth	Surface
SIS1a	Circular	Colorless	Entire	Convex	Glistening	Surface
SIS1b	Circular	Colorless	Entire	Convex	Glistening	Surface
SIS2a	Circular	Creamy	Entire	Convex	Smooth	Surface
SIS2b	x	x	x	x	x	x
SIS3a	Circular	Pink	Entire	Flat	Smooth	Surface
SIS3b	Circular	Creamy	Entire	Convex	Smooth	Surface
SIS4a	Circular	White	Entire	Convex	Glistening	Surface
SIS4b	Circular	Yellow	Entire	Convex	Glistening	Surface
SIS5a	Circular	Purple	Entire	Flat	Smooth	Surface
SIS5b	Circular	Creamy	Entire	Convex	Smooth	Surface
TIS_{1a}	Circular	Yellow	Entire	Flat	Rough	Surface
TIS_{1b}	x	x	x	x	x	Surface
TIS_{2a}	x	x	x	x	x	Surface
TIS_{2b}	Circular	Yellow	Entire	Flat	Glistening	Surface
$TIS_{3(a+b)}$	Circular	Yellow	Entire	Flat	Smooth	Surface
$TIS_{4(a+b)}$	x	x	x	x	x	x
TIS_{5a}	x	x	x	x	x	Surface
TIS_{5b}	Circular	Yellow	Entire	Flat	Glistening	Surface

x = No growth, NMS= Nutrient Agar Mucus Sample, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample.

According to the table no. 9 from the intestine of Open Water Tilapia -

Total strains were 42 and growths were not found in 6 strains, growths were observed in 36 strains. 35 circular colonies and 1 irregular colony were shown. Colonies of different colors were chosen such as creamy, yellow, red, pale yellow, pink, white, purple, brown, colorless and gray.32 colonies showed entire margin, 3 irregular margins and 1 colony showed undulate margin. 19 colonies showed convex surface elevation, 14 colonies showed flat surface elevation and 3colonies showed raised elevation. 21 colonies showed smooth surface, 1 showed rough surface and 14 colonies showed glistening surface .All growth were observed on agar surface.

Table-10: Morphological characteristics of bacterial cells isolated from the intestine of Open water Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
NIS_{1a}	-	Short rod	Arranged in small chain
NIS_{1b}	-	Rod	Arranged in single and pair
NIS_{2a}	-	Rod	Arranged in long chain
NIS_{2b}	-	Rod	Arranged in single and pair
NIS_{3a}	+	Coccus	Scattered Arrangement
NIS_{3b}	-	Rod	Arranged in single and pair
NIS_{4a}	-	Rod	Arranged in single and pair
NIS_{4b}	-	Rod	Arranged in chain
NIS_{5a}	-	Rod	Arranged in single and pair
NIS_{5b}	+	Rod	Arranged in single and pair
MIS_{1a}	-	Rod	Arranged in single and pair
MIS_{1b}	-	Rod	Arranged in single cell
MIS_{1c}	-	Rod	Arranged in single and pair
MIS_{2a}	-	Short rod	Arranged in small chain
MIS_{2b}	-	Rod	Arranged in single and pair
MIS_{3a}	-	Rod	Arranged in chain form
MIS_{3b}	-	Rod	Arranged in single and pair
MIS_{3c}	-	Rod	Arranged in single and pair
MIS_{4a}	-	Rod	Arranged in single and pair
MIS_{4b}	-	Rod	Arranged in single and pair
MIS_{5a}	-	Rod	Arranged in single and pair
MIS_{5b}	-	Rod	Arranged in single and pair
SIS_{1a}	-	Rod	Arranged in single and pair
SIS_{1b}	-	Rod	Arranged in single and pair
SIS_{2a}	-	Rod	Arranged in single and pair
SIS_{2b}	x	x	x
SIS_{3a}	-	Rod	Arranged in single and pair
SIS_{3b}	-	Short rod	Arranged in small chain
SIS_{4a}	-	Short rod	Arranged in small chain
SIS_{4b}	-	Rod	Arranged in single and pair
SIS_{5a}	-	Rod	Arranged in long chain
SIS_{5b}	-	Rod	Arranged in single and pair
TIS_{1a}	-	Rod	Arranged in single and pair
TIS_{1b}	x	x	x
TIS_{2a}	x	x	x
TIS_{2b}	-	Rod	Arranged in single and pair
$TIS_{3(a+b)}$	-	Rod	Arranged in single and pair
$TIS_{4(a+b)}$	x	x	x
TIS_{5a}	x	x	x
TIS_{5b}	-	Rod	Arranged in single and pair

x = No growth, NMS= Nutrient Agar Mucus Sample, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample.

According to table no 10 from the intestine of Open Water Tilapia -

Total strains were 42. But growths were observed in 36 strains and no growth found in 6 strains. All of them, 31 isolates were rod, 4 were short rod and1was coccus . Out of 36, 35 isolates were Gram negative and1 was gram positive. Among them 26 were arranged in single and pair, 2 were long chain, 1was single cell, 2 were long chain, 4 were small chain and 1 was arranged in scattered form.

Table-11: Biochemical characteristics of gram negative bacterial cells isolated from the intestine of Open Water Tilapia (*Oreochromis niloticus*) grown on different media (MacConkey, SS and TCBS)

Stain	Gram reaction	Slant	Butt	Gas	Motility	Indole	Urease	Catalase	Oxidase	Isolates
MIS_{1a}	-	K	A	-	+	+	-	+	+	*Plesimonas*
MIS_{1b}	-	A	A	+	+	-	-	+	-	*E.coli*
MIS_{1c}	-	A	A	+	+	-	-	+	-	*Enterobacter*
MIS_{2a}	-	K	A	-	+	+	-	+	+	*Pseudomonas*
MIS_{2b}	-	A	A	-	-	-	-	-	-	Unidentified
MIS_{3a}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MIS_{3b}	-	K	A	-	+	+	-	+	-	*Pseudomonas*
MIS_{3c}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MIS_{4a}	-	A	A	+	+	-	-	+	-	*E.coli*
MIS_{4b}	-	K	A	-	+	+	-	+	+	*Pseudomonas*
MIS_{5a}	-	K	A	-	-	-	+	+	-	*Yersinia*
MIS_{5b}	-	K	A	+	+	-	-	+	-	*Enterobacter*
SIS_{1a}	-	K	A	-	-	-	-	+	-	*Shigella*
SIS_{1b}	-	K	A	+	+	-	-	+	-	*Salmonella*
SIS_{2a}	-	K	A	+	+	-	-	+	-	*Salmonella*
SIS_{2b}	x	x	x	x	x	x	x	x	x	x
SIS_{3a}	-	A	A	-	-	-	-	+	+	Unidentified
SIS_{3b}	-	K	A	-	-	-	-	+	-	*Shigella*
SIS_{4a}	-	K	A	-	-	-	-	+	-	*Shigella*
SIS_{4b}	-	K	A	+	-	+	+	+	-	Unidentified
SIS_{5a}	-	A	A	+	+	-	-	+	-	Unidentified
SIS_{5b}	-	K	A	+	+	-	-	+	-	*Salmonella*
TIS_{1a}	-	K	A	-	+	+	-	+	+	*Vibrio*
TIS_{1b}	x	x	x	x	x	x	x	x	x	x
TIS_{2a}	x	x	x	x	x	x	x	x	x	x
TIS_{2b}	-	K	A	-	+	+	-	+	+	*Vibrio*
$TIS_{3(a+b)}$	-	K	A	-	+	+	-	+	+	*Vibrio*
$TIS_{4(a+b)}$	x	x	x	x	x	x	x	x	x	x
TIS_{5a}	x	x	x	x	x	x	x	x	x	x
TIS_{5b}	-	K	A	-	+	+	-	+	+	*Vibrio*

x = No growth, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample, K = Alkaline (red) reaction, A= Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction

According to table no 11 from the intestine of Open Water Tilapia -

Total strains were 32.Growth was observed in 26 strains and growths were not found in 6 strains. KIA test indicated that out of 26 strains, 6 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 20 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 26 strains, 12 produced gases which were observed by formation of bubbles in the media and 14 strains were not produced gas.

MIU test indicated that out of 26 strains, 19 were motile and 7 were non- motile. Iodole test were positive for 12 rods and 14 strains were not positive for Iodole test. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains only 2 showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test: All the isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test: While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 26 gram negative rods, 11 were oxidase positive and 15 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table-12: Cultural characteristics of bacterial cells isolated from mucus of Cultured Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Colony shape	Color	Margin	Elevation	Surface	Growth on Agar
NMS_{1a}	Circular	Creamy	Undulate	Convex	Smooth	Surface
NMS_{1b}	Circular	Purple	Entire	Convex	Dry	Surface
NMS_{2a}	Circular	Creamy	Entire	Convex	Smooth	Surface
NMS_{2b}	x	x	x	x	x	x
NMS_{3a}	Circular	Pale yellow	Undulate	Raised	Smooth	Surface
NMS_{3b}	Circular	Creamy	Entire	Convex	Powdery	Surface
NMS_{4a}	Circular	White	Entire	Flat	x	Surface
NMS_{4b}	x	x	x	x	Smooth	x
NMS_{5a}	x	x	x	x	Glistening	x
NMS_{5b}	Circular	Purple	`Entire	Convex	Smooth	Surface
MMS_{1a}	Circular	Creamy	Undulate	Flat	Smooth	Surface
MMS_{1b}	Circular	White	Entire	Convex	Smooth	Surface
MMS_{2a}	x	x	x	x	x	x
MMS_{2b}	Circular	Purple	Entire	Convex	Rough	Surface
MMS_{3a}	x	x	x	x	x	x
MMS_{3b}	Circular	Pale yellow	Entire	Convex	Smooth	Surface
MMS_{4a}	Circular	Creamy	Entire	Convex	Smooth	Surface
MMS_{4b}	x	x	x	x	x	x
MMS_{5a}	x	x	x	x	x	x
MMS_{5b}	Circular	Creamy	Entire	Raised	Dry	Surface
SMS_{1a}	Circular	Purple	Entire	Convex	Smooth	Surface
SMS_{1b}	x	x	x	x	x	x
SMS_{2a}	Circular	Creamy	Entire	Convex	Smooth	Surface
SMS_{2b}	Circular	Purple	Entire	Convex	Smooth	Surface
SMS_{3a}	Circular	Pink	Entire	Flat	Smooth	Surface
SMS_{3b}	x	x	x	x	x	x
SMS_{4a}	x	x	x	x	x	x
SMS_{4b}	Circular	Creamy	Entire	Flat	Dry	Surface
SMS_{5a}	Circular	Creamy	Undulate	Convex	Dry	Surface
SMS_{5b}	Circular	Pink	Entire	Convex	Smooth	Surface
$TMS_{1(a+b)}$	x	x	x	x	x	x
$TMS_{2(a+b)}$	x	x	x	x	x	x
$TMS_{3(a+b)}$	x	x	x	x	x	x
$TMS_{4(a+b)}$	x	x	x	x	x	x
$TMS_{5(a+b)}$	x	x	x	x	x	x

x = No growth, NMS= Nutrient Agar Mucus Sample, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample.

According to the table no. 12 from mucus of Cultured Tilapia –

Total strains were 40 and no growths were found in 20 strains. 20 circular colonies were shown. Colonies of different colors were chosen such as creamy, pale yellow, white, pink and purple.16 colonies showed entire margin and 4 colonies showed undulate margin. 14 colonies showed convex elevation, 4 colonies showed flat elevation and 2 colonies showed raised elevation. 14 colonies showed smooth surface, 1 showed rough surface and 4 showed dry surface, 1 showed powdery surface and 1 showed glistening surface .All growth were observed on agar surface.

Table-13: Morphological characteristics of bacterial cells isolated from mucus of Cultured Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
NMS_{1a}	-	Rod	Arranged in single and pair
NMS_{1b}	-	Rod	Arranged in single cell
NMS_{2a}	-	Rod	Arranged in long chain
NMS_{2b}	x	x	x
NMS_{3a}	-	Short rod	Arranged in small chain
NMS_{3b}	-	Rod	Arranged in long chain
NMS_{4a}	-	Rod	Arranged in single and pair
NMS_{4b}	x	x	x
NMS_{5a}	x	x	x
NMS_{5b}	-	Rod	Arranged in single cell
MMS_{1a}	-	Rod	Arranged in long chain
MMS_{1b}	-	Rod	Arranged in single and pair
MMS_{2a}	x	x	x
MMS_{2b}	-	Short rod	Arranged in small chain
MMS_{3a}	x	x	x
MMS_{3b}	-	Rod	Arranged in single cell
MMS_{4a}	x	x	x
MMS_{4b}	-	Short rod	Arranged in small chain
MMS_{5a}	x	x	x
MMS_{5b}	-	Rod	Arranged in single and pair
SMS_{1a}	x	x	x
SMS_{1b}	-	Rod	Arranged in single cell
SMS_{2a}	-	Rod	Arranged in long chain
SMS_{2b}	-	Rod	Arranged in single and pair
SMS_{3a}	-	Rod	Arranged in single cell
SMS_{3b}	x	x	x
SMS_{4a}	x	x	x
SMS_{4b}	-	Short rod	Arranged in small chain
SMS_{5a}	-	Rod	Arranged in single and pair
SMS_{5b}	-	Short rod	Arranged in single cell
$TMS_{1(a+b)}$	x	x	x
$TMS_{2(a+b)}$	x	x	x
$TMS_{3(a+b)}$	x	x	x
$TMS_{4(a+b)}$	x	x	x
$TMS_{5(a+b)}$	x	x	x

x = No growth, NMS= Nutrient Agar Mucus Sample, MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample.

According to table no 13 from mucus of Cultured Tilapia-

Total strains were 40. But growths were observed in 20 strains and no growth found in 20 strains. All of them, 15 isolates were rod and gram negative and 5 were short rod and gram negative. Among them 6 were arranged in single and pair, 4 were in small chain, 4 were long chain and 6 were single cell.

Table-14: Biochemical characteristics of gram negative bacterial cells isolated from mucus of Cultured Tilapia (*Oreochromis niloticus*) grown on different media (MacConkey, SS and TCBS*)

Stain	Gram reaction	Slant	Butt	Gas	Motility	Indole	Urease	Catalase	Oxidase	Isolates
MMS_{1a}	-	A	A	+	+	-	-	+	-	*E.coli*
MMS_{1b}	-	K	A	-	-	-	-	+	-	Unidentified
MMS_{2a}	x	x	x	x	x	x	x	x	x	x
MMS_{2b}	-	A	A	+	-	+	-	+	-	*Klebsiella*
MMS_{3a}	x	x	x	x	x	x	x	x	x	x
MMS_{3b}	-	K	A	-	+	+	-	+	+	*Plesiomonas*
MMS_{4a}	x	K	A	-	+	+	-	+	+	*Pesudomonas*
MMS_{4b}	-	x	x	x	x	x	x	x	x	x
MMS_{5a}	x	x	x	x	x	x	x	x	x	x
MMS_{5b}	-	K	A	+	+	+	-	+	+	*Aeromonas*
SMS_{1a}	-	A	A	+	+	-	-	+	-	*E.coli*
SMS_{1b}	x	x	x	x	x	x	x	x	x	x
SMS_{2a}	-	A	A	+	+	-	-	+	-	*E.coli*
SMS_{2b}	-	K	A	-	-	+	-	+	-	Unidentified
SMS_{3a}	-	K	A	+	+	+	-	+	+	Unidentified
SMS_{3b}	x	x	x	x	x	x	x	x	x	x
SMS_{4a}	x	x	x	x	x	x	x	x	x	x
SMS_{4b}	-	A	A	+	+	-	-	+	-	*E.coli*
SMS_{5a}	-	A	A	+	+	-	-	+	-	*E.coli*
SMS_{5b}	-	K	A	+	+	+	+	+	+	Unidentified

x = No growth *TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Mucus Sample, no growth was obsearved , MMS= MacConkey Agar Mucus Sample, SMS= Salmonella- Shigella Agar Mucus Sample K = Alkaline (red) reaction, A= Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction

According to table no 14 from mucus of Cultured Tilapia –

Total strains were 20.Growth was observed in 13strains and growths were not found in 7 strains. KIA test indicated that out of 13 strains, 6 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow.

7 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 13 strains, 9 produced gases which were observed by formation of bubbles in the media and 4 were not produced gas.

MIU test indicated that out of 13 strains, 10 strains were motile and 3 were non-motile. Iodole test were positive for 7 rods and 6 were indole negative. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains only 1 showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test: All the isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 13 gram negative rods, 5 were oxidase positive and 8were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table 15: Cultural characteristics of bacterial cells isolated from the intestine of cultured Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Colony shape	Color	Margin	Elevation	Surface	Growth on Agar
NIS_{1a}	Circular	White	Entire	Convex	Smooth	Surface
NIS_{1b}	Circular	Yellow	Entire	Convex	Rough	Surface
NIS_{2a}	Circular	Creamy	Entire	Convex	Smooth	Surface
NIS_{2b}	x	x	x	x	x	x
NIS_{3a}	Circular	Pale yellow	Entire	Convex	Smooth	Surface
NIS_{3b}	Circular	Creamy	Entire	Convex	Powdery	Surface
NIS_{4a}	x	x	x	x	x	x
NIS_{4b}	Circular	Creamy	Entire	Flat	Smooth	Surface
NIS_{5a}	Circular	Pale yellow	Entire	Convex	Glistening	Surface
NIS_{5b}	Irregular	White	Undulate	Raised	Smooth	Surface
MIS_{1a}	Circular	Purple	Undulate	Convex	Smooth	Surface
MIS_{1b}	x	x	x	x	x	x
MIS_{2a}	Circular	Pale yellow	Entire	Convex	Smooth	Surface
MIS_{2b}	Circular	Pink	Entire	Raised	Smooth	Surface
MIS_{3a}	x	x	x	x	x	x
MIS_{3b}	Circular	Creamy	Entire	Convex	Smooth	Surface
MIS_{4a}	Circular	Pink	Entire	Flat	Smooth	Surface
MIS_{4b}	Circular	Yellow	Entire	Convex	Smooth	Surface
MIS_{5a}	Circular	Creamy	Undulate	Convex	Smooth	Surface
MIS_{5b}	x	x	x	x	x	x
SIS_{1a}	x	x	x	x	x	x
SIS_{1b}	Irregular	Creamy	Entire	Convex	Smooth	Surface
SIS_{2a}	Circular	Creamy	Entire	Convex	Rough	Surface
SIS_{2b}	x	x	x	x	x	x
SIS_{3a}	Circular	Pink	Entire	Convex	Glistening	Surface
SIS_{3b}	x	x	x	x	x	x
SIS_{4a}	Circular	Yellow	Entire	Convex	Smooth	Surface
SIS_{4b}	Circular	Purple	Entire	Flat	Smooth	Surface
SIS_{5a}	x	x	x	x	x	x
SIS_{5b}	Circular	White	Entire	Convex	Smooth	Surface
$TIS_{1(a+b)}$	x	x	x	x	x	x
$TIS_{2(a+b)}$	x	x	x	x	x	x
$TIS_{3(a+b)}$	x	x	x	x	x	x
$TIS_{4(a+b)}$	x	x	x	x	x	x
$TIS_{5(a+b)}$	x	x	x	x	x	x

x = No growth, NMS= Nutrient Agar Intestine Sample, MMS= MacConkey Agar Intestine Sample, SMS= Salmonella- Shigella Agar Agar Intestine Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Agar Intestine Sample.

According to table no 15 from intestine of Cultured Tilapia -

Total strain was 40 and no growth was found in 19 strains. 19 circular colonies and 2 irregular colonies are shown. Colonies of different colors were chosen such as creamy, yellow, pale yellow, pink, white and purple .18 colonies showed entire margin, and 3 colonies showed undulate margin. 16 colonies showed convex elevation, 3 colonies showed flat elevation and 2 colonies showed raised elevation. 14 colonies showed smooth surface, 1 showed rough surface and 4 showed dry surface, 1 showed powdery surface and 1 showed glistening surface .All growth was observed on agar surface.

Table-16: Morphological characteristics of bacterial cells isolated from the intestine of Cultured Tilapia (*Oreochromis niloticus*) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
NIS_{1a}	-	Rod	Arranged in single cell
NIS_{1b}	+	Coccus	Scattered arrangement
NIS_{2a}	-	Rod	Arranged in long chain
NIS_{2b}	x	x	x
NIS_{3a}	-	Short rod	Arranged in small chain
NIS_{3b}	-	Rod	Arranged in single and pair
NIS_{4a}	-	Rod	Arranged in single and pair
NIS_{4b}	x	x	x
NIS_{5a}	-	Rod	Arranged in long chain
NIS_{5b}	-	Rod	Arranged in single and pair
MIS_{1a}	-	Short rod	Arranged in small chain
MIS_{1b}	x	x	x
MIS_{2a}	-	Rod	Arranged in single and pair
MIS_{2b}	-	Rod	Arranged in single cell
MIS_{3a}	-	Rod	Arranged in long chain
MIS_{3b}	x	x	x
MIS_{4a}	-	Short rod	Arranged in small chain
MIS_{4b}	-	Rod	Arranged in single and pair
MIS_{5a}	-	Rod	Arranged in single cell
MIS_{5b}	x	x	x
SIS_{1a}	x	x	x
SIS_{1b}	-	Short rod	Arranged in small chain
SIS_{2a}	-	Rod	Arranged in single and pair
SIS_{2b}	x	x	x
SIS_{3a}	-	Rod	Arranged in single cell
SIS_{3b}	x	x	x
SIS_{4a}	-	Rod	Arranged in single cell
SIS_{4b}	-	Rod	Arranged in long chain
SIS_{5a}	x	x	x
SIS_{5b}	-	Short rod	Arranged in small chain
$TIS_{1(a+b)}$	x	x	x
$TIS_{2(a+b)}$	x	x	x
$TIS_{3(a+b)}$	x	x	x
$TIS_{4(a+b)}$	x	x	x
$TIS_{5(a+b)}$	x	x	x

x = No growth, NMS= Nutrient Agar Intestine Sample, MMS= MacConkey Agar Intestine Sample, SMS= Salmonella- Shigella Agar Agar Intestine Sample, TMS= Thio Sulfate Citrate Bile salts Sucrose Agar Agar Intestine Sample.

According to table no 16 from the intestine of Cultured Tilapia –

Total strains were 40. But growth was observed in 21strains and no growth found in 19 strains. All of them, 15 isolates were rod and were gram negative. 5 were short rod and gram negative, 1 was coccus and gram positive. Among them 6 were arranged in single and pair, 5 were in single cell, 5 were small chain, 4 were long chain and 1was arranged scattered form.

Table-17: Biochemical characteristics of gram negative bacterial cells isolated from the intestine of Cultured Tilapia (*Oreochromis niloticus*) grown on differen media (MacConkey, SS and TCBS*).

Stain	Gram reaction	Slant	Butt	Gas	Motility	Indole	Urease	Catalase	Oxidase	Isolates
MIS_{1a}	-	K	A	-	-	-	-	+	-	Unidentified
MIS_{1b}	x	x	x	x	x	x	x	x	x	x
MIS_{2a}	-	K	A	-	+	+	-	+	+	*Plesiomonas*
MIS_{2b}	-	A	A	+	+	-	-	+	-	*E.coli*
MIS_{3a}	x	x	x	x	x	x	x	x	x	x
MIS_{3b}	-	K	A	-	-	-	-	+	-	Unidentified
MIS_{4a}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MIS_{4b}	-	A	A	-	-	-	-	-	-	Unidentified
MIS_{5a}	-	K	A	-	+	+	-	+	+	*Pseudomonas*
MIS_{5b}	x	x	x	x	x	x	x	x	x	x
SIS_{1a}	x	x	x	x	x	x	x	x	x	x
SIS_{1b}	-	K	A	-	-	-	-	+	-	*Shigella*
SIS_{2a}	-	K	A	+	+	-	-	+	-	*Salmonella*
SIS_{2a}	x	x	x	x	x	x	x	x	x	x
SIS_{3a}	-	K	A	-	-	-	-	+	-	*Shigella*
SIS_{5b}	x	x	x	x	x	x	x	x	x	x
SIS_{4a}	-	K	A	+	-	+	+	+	-	Unidentified
SIS_{4b}	-	A	A	+	+	-	-	+	-	Unidentified
SIS_{5a}	x	x	x	x	x	x	x	x	x	x
SIS_{5b}	-	K	A	+	+	+	-	+	+	Unidentified

x = No growth, MMS= MacConkey Agar Intestine Sample, SMS= Salmonella- Shigella Agar Agar Intestine Sample, K = Alkaline (red) reaction, A = Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction, * No growth was observed in TCBS* agar.

According to table no 17 from intestine of Cultured Tilapia -

Total strain was 20.Growth was observed in 13 strains and growth was not found in 7 strains. KIA test indicate that out of 13 strains, 4 strains gave positive

results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 9strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 13strains, 7 produced gas which was observed by formation of bubbles in the media and 6 not produced gas.

MIU test indicated that out of 13 strains, 6 were motile and 7 were non- motile. Iodole test were positive for 8 rods and 5 were indole negative. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains only 1 showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test: Out of 13 strains, 1 isolates was unidentified, that showed negative catalase. All other isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 13 gram negative rods, 4 were oxidase positive and 9 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table-18: Comparative analysis of bacterial present in mucus and intestine of both Open water and cultured fish:

Name of the bacteria	Mucus		Intestine	
	Open Water	Culture	Open Water	Culture
Shigella	+	-	+	+
Salmonella	+	-	+	+
Pseudomonas	+	+	+	+
Plesiomonas	+	+	+	+
Aeromonas	+	+	+	+
Klebseilla	+	+	+	+
E.coli	+	+	+	+
Vibrio	+	-	+	-
Yersinia	+	-	+	-
Enterobacter	+	-	+	-

+= Presence, - = Absence

From the table no 18, it was observed that in mucus and intestine of Open Water fish *Shigella, Salmonella, Pseudomonas, Plesiomonas, Aeromonas, Klebseilla, E.coli, Vibrio, Yersinia, Enterobacter* were present while in case of mucus of cultured fish *Vibrio, Yersinia, Enterobacter* were absent and in intestine of cultured fish *Vibrio, Yersinia, Enterobacter, , Klebseilla* were absent .

Table-19: Total bacterial count of the rearing water samples of the Open Water (Lake) grown on Nutrient and MacConky agar

Water sample	Nutrient agar(cfu/ml)	Average (cfu/g)	MacConkey agar (cfu/ml)	Average (cfu/ml)
Sample 1	1.2×10^5	1.75×10^5	1.1×10^5	0.96×10^5
Sample 2	1.9×10^5		0.9×10^5	
Sample 3	2.16×10^5		0.9×10^5	

The total viable bacterial count in the rearing water samples of Open Water Tilapia (lake) on Nutrient and MacConkey agar were presented in table no 19. From the table on Nutrient agar the average bacterial counts were 1.75×10^5 (cfu/ml) where on MacConkey agar the bacterial counts were 0.96×10^5 (cfu/ml).

Table-20: Total bacterial count of the rearing water samples of the Cultured Pond grown on Nutrient and MacConky agar

Water Sample	Nutrient Agar(cfu/ml)	Average (cfu/ml)	MacConkey Agar(cfu/ml)	Average (cfu/ml)
Sample 1	$1.50x10^4$	$1.45x10^4$	$1.40x10^4$	$1.12x10^4$
Sample 2	$2.25x10^4$		$1.50x10^4$	
Sample 3	$0.7x10^4$		$0.7x10^4$	

The total viable bacterial count in the rearing water samples of the pond (cultured) on Nutrient and MacConkey agar were presented in table no 20. From the table 20 on Nutrient agar the average bacterial counts were 1.45×10^4(cfu/ml) where on MacConkey agar the bacterial counts were 1.10×12^4 (cfu/ml).

Table -21: Total bacterial count of the rearing water samples of Cultured Pond and Open Water (Lake) grown on Nutrient and MacConkey agar.

Type of water	Nutrient Agar(cfu/ml)	MacConkey Agar(cfu/ml)
Pond	1.45×10^4	1.12×10^4
Open Water (Lake)	1.75×10^5	0.96×10^5

The total viable bacterial count in the rearing water samples of the Cultured Pond and Open Water (lake) on Nutrient and MacConkey agar is presented in table no 21. From the table 21 on Nutrient agar the average bacterial counts were 1.45×10^4(cfu/ml) where on MacConkey agar the bacterial counts were 1.12×10^4 (cfu/ml).On Nutrient agar the average bacterial counts were 1.75×10^5(cfu/ml) where on MacConkey agar the bacterial counts were 0.96×10^5 (cfu/ml).

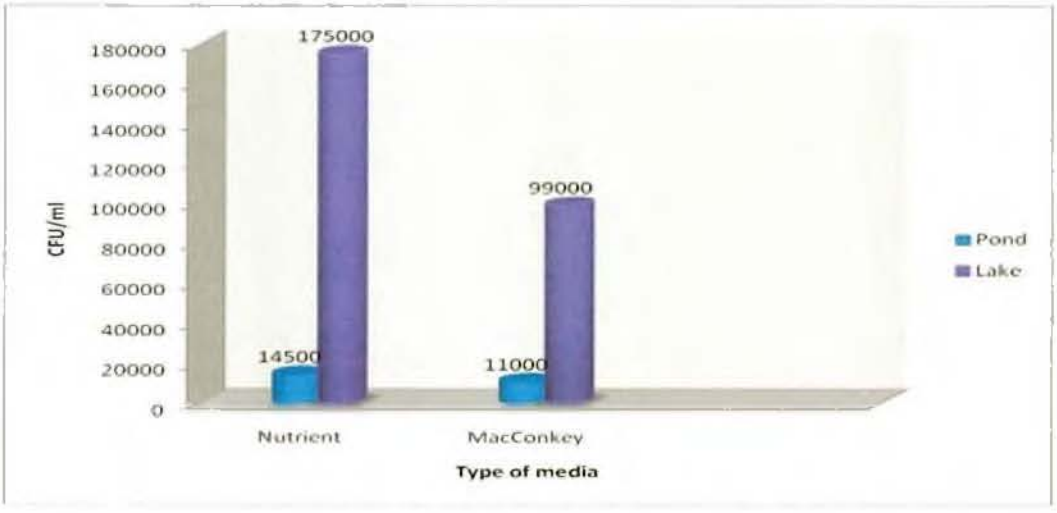


Figure 2: Comparison Total bacterial counts (cfu/ml) of the rearing water samples of Cultured Pond and Open Water (Lake) grown on Nutrient and MacConkey agar

Table-22: Cultural characteristics of bacterial cells isolated from the rearing water samples of Open Water (lake) grown on different culture media (Nutrient, MacConkey, SS and TCBS).

Strain	Colony shape	Colour	Margin	Elevation	Surface	Growth on Agar
NWS_{1a}	Circular	Creamy	Entire	Convex	Smooth	Surface
NWS_{1b}	Circular	Creamy	Entire	Flat	Glistening	Surface
NWS_{2a}	Circular	Purple	Entire	Convex	Glistening	Surface
NWS_{2b}	Circular	Pink	Entire	Convex	Smooth	Surface
NWS_{3a}	Circular	Creamy	Entire	Raised	Smooth	Surface
NWS_{3b}	Circular	LightCreamy	Entire	Convex	Glistening	Surface
NWS_{3c}	Circular	Pink	Entire	Flat	Glistening	Surface
MWS_{1a}	Circular	Pink	Irregular	Flat	Smooth	Surface
MWS_{1b}	Circular	Creamy	Entire	Convex	Smooth	Surface
MWS_{2a}	Circular	Colourless	Entire	Flat	Dry	Surface
MWS_{2b}	Circular	Creamy	Entire	Convex	Dry	Surface
MWS_{2c}	Circular	Creamy	Entire	Convex	Dry	Surface
MWS_{3a}	Circular	Pink	Entire	Raised	Smooth	Surface
MWS_{3b}	Circular	Pale Yellow	Entire	Convex	Smooth	Surface
SWS_{1a}	Circular	Creamy	Entire	Convex	Powdery	Surface
SWS_{1b}	Circular	Creamy	Entire	Flat	Powdery	Surface
SWS_{2a}	Circular	Colourless	Entire	Flat	Smooth	Surface
SWS_{2b}	Circular	Creamy	Entire	Convex	Smooth	Surface
SWS_{3a}	Circular	Colourless	Entire	Convex	Smooth	Surface
SWS_{3b}	x	x	x	x	x	x
TWS_{1a}	Circular	Yellow	Entire	Flat	Smooth	Surface
TWS_{1b}	Circular	Yellow	Entire	Flat	Smooth	Surface
$TWS_{2(a+b)}$	x	x	x	x	x	x
TWS_{3a}	Circular	Yellow	Entire	Flat	Smooth	Surface
TWS_{3b}	x	x	x	x	x	x

x = No growth, NWS= Nutrient Agar Water Sample, MWS= MacConkey Agar Water Sample, SWS= Salmonella- Shigella Agar Water Sample, TWS= Thio Sulfate Citrate Bile salts Sucrose Agar Water Sample.

According to table No 22, from the rearing water samples of Open Water (lake) on -

Total strains were 26. Growths were observed in 22 strains and growths were not found in 4 strains. 22 circular colonies were shown. Colonies of different colors were chosen such as light creamy, creamy yellow, pale yellow, pink, white,

colorless and purple .21 colonies showed entire margin, and 1colony showed irregular margin. 11 colonies showed convex surface elevation, 9 colonies showed flat surface elevation and 2 colonies showed raised elevation. 13 colonies showed smooth surface, 3 showed dry surface, 2 showed powdery surface and 4 showed glistening surface .All growth were observed on agar surface.

Table-23: Morphological characteristics of bacterial cells isolated from the rearing water samples of Open Water (lake) grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
NWS_{1a}	-	Rod	Arranged in single cell
NWS_{1b}	-	Rod	Arranged in long chain
NWS_{2a}	-	Rod	Arranged in small chain
NWS_{2b}	-	Rod	Arranged in long chain
NWS_{3a}	-	Short rod	Arranged in small chain
NWS_{3b}	-	Rod	Arranged in long chain
NWS_{3c}	-	Rod	Arranged in single and pair
MWS_{1a}	-	Rod	Arranged in single and pair
MWS_{1b}	-	Short Rod	Arranged in small chain
MWS_{2a}	-	Rod	Arranged in long chain
MWS_{2b}	-	Short Rod	Arranged in small chain
MWS_{2c}	-	Short Rod	Arranged in small chain
MWS_{3a}	-	Rod	Arranged in single cell
MWS_{3b}	-	Rod	Arranged in single and pair
SWS_{1a}	-	Rod	Arranged in single and pair
SWS_{1b}	-	Rod	Arranged in chain
SWS_{2a}	-	Rod	Arranged in chain
SWS_{2b}	-	Rod	Arranged in chain
SWS_{3a}	-	Rod	Arranged in single and pair
SWS_{3b}	x	x	x
TWS_{1a}	-	Curved Rod	Arranged in single and pair
TWS_{1b}	-	Curved Rod	Arranged in single and pair
$TWS_{2(a+b)}$	x	x	x
TWS_{3a}	-	Curved Rod	Arranged in single and pair
TWS_{3b}	x	x	x

x = No growth, NWS= Nutrient Agar Water Sample, MWS= MacConkey Agar Water Sample, SWS= Salmonella- Shigella Agar Water Sample, TWS= Thio Sulfate Citrate Bile salts Sucrose Agar Water Sample.

According to table no 23 from the rearing water samples of Open Water (lake) -

Total strains were 26. But growth were observed in 22 strains and growth not found in 4 strains. All of them, 16 isolates were rod and gram negative and 3 were short rod and gram negative.3 were curved rod and grain negative. Among them 10 were arranged in single and pair, 4 were in small chain, 4 were long chain, 3 were chain, 1 was short chain, 2 were in single cell.

Table-24: Biochemical characteristics of gram negative bacterial cells isolated from the Rearing water samples of Open Water (lake) grown on different mediaMacConkey, SS and TCBS)

Stain	Gram reaction	Slant	Butt	Gas	Motility	Indole	Urease	Catalase	Oxidase	Isolates
MWS_{1a}	-	A	A	+	+	-	-	+	-	*Enterobacter*
MWS_{1b}	-	A	A	+	-	+	+	+	-	*Klebsiella*
MWS_{2a}	-	K	A	-	+	+	-	+	+	*Pscudomonas*
MWS_{2b}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MWS_{2c}	-	K	A	-	-	-	+	+	-	*Yersinia*
MWS_{3a}	-	A	A	+	+	-	-	+	-	*E.coli*
MWS_{3b}	-	K	A	-	+	+	-	+	+	*Plesiomonas*
SWS_{1a}	-	K	A	+	+	-	-	+	-	*Salmonella*
SWS_{1b}	-	K	K	-	-	+	+	-	-	Unidentified
SWS_{2a}	-	K	A	-	+	+	-	+	+	Unidentified
Sws_{2b}	-	K	A	+	+	-	-	+	-	*Salmonella*
SWS_{3a}	-	K	A	-	-	-	-	+	-	*Shigella*
SWS_{3b}	x	x	x	x	x	x	x	x	x	x
TWS_{1a}	-	K	A	-	+	+	-	+	+	*Vibrio*
TWS_{1b}	-	K	A	-	+	+	-	+	+	*Vibrio*
$TWS_{2(a+b)}$	x	x	x	x	x	x	x	x	x	x
TWS_{3a}	-	K	A	-	+	+	-	+	+	*Vibrio*
TWS_{3b}	x	x	x	x	x	x	x	x	x	x

x = No growth, NWS= Nutrient Agar Water Sample, MWS= MacConkey Agar Water Sample,SWS= Salmonella- Shigella Agar Water Sample, TWS= Thio Sulfate Citrate Bile salts Sucrose Agar Water Sample. K = Alkaline (red) reaction, A = Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction

According to table no 24 from the rearing water samples of the lake –

Total strains were 19. Growths were observed in 15 strains and growths were not observed in 4 strains. KIA test indicated that out of 15 strains, 3 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 12 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 15 strains, 6 produced gases which were observed by formation of bubbles in the media. 9 strains were not produced gas.

MIU test indicated that out of 15 strains, 9 strains were motile and 6 were non-motile. Iodole test were positive for 12 rods. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. 3 strains were non motile. Among the strains 2 showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test: Out of 14 strains, 13 were showed positive catalase test.Only 1 strain was showed negative catalase test. All other isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 15 gram negative rods, 6 were oxidase positive and 9 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table-25: Cultural characteristics of bacterial cells isolated from the rearing water Samples of the Cultured Pond grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Colony shape	Colour	Margin	Elevation	Surface	Growth on Agar
NWS_{1a}	Circular	Creamy	Entire	Convex	Dry	Surface
NWS_{1b}	Circular	Creamy	Entire	Flat	Powdery	Surface
NWS_{2a}	Circular	Purple	Entire	Convex	Smooth	Surface
NWS_{2b}	Circular	Pink	Entire	Convex	Smooth	Surface
NWS_{3a}	Circular	Creamy	Entire	Raised	Smooth	Surface
NWS_{3b}	Circular	Creamy	Entire	Convex	Smooth	Surface
MWS_{1a}	Circular	White	Entire	Convex	Smooth	Surface
MWS_{1b}	Circular	Purple	Entire	Raised	Smooth	Surface
MWS_{2a}	Circular	Creamy	Entire	Convex	Smooth	Surface
MWS_{2b}	Circular	Pink	Entire	Convex	Smooth	Surface
MWS_{3a}	Circular	Yellow	Entire	Convex	Glistening	Surface
MWS_{3b}	x	x	x	x	x	x
SWS_{1a}	Circular	Pale orange	Entire	Convex	Glistening	Surface
SWS_{1b}	x	x	x	x	x	x
SWS_{2a}	x	x	x	x	x	x
SWS_{2b}	Circular	Creamy	Entire	Convex	Glistening	Surface
SWS_{3a}	Irregular	Creamy	Entire	Convex	Glistening	Surface
SWS_{3b}	Circular	Creamy	Entire	Raised	Glistening	Surface
$TWS_{1(a+b)}$	x	x	x	x	x	x
$TWS_{2(a+b)}$	x	x	x	x	x	x
$TWS_{3(a+b)}$	x	x	x	x	x	x

x = No growth, NWS= Nutrient Agar Water Sample, MWS= MacConkey Agar Water Sample, SWS= Salmonella- Shigella Agar Water Sample, TWS= Thio Sulfate Citrate Bile salts Sucrose Agar Water Sample.

According to table No 25, from the rearing water samples of the Cultured Pond on -

Total strains were 24. Growths were observed in 15 strains and growths were not found in 9 strains. 14 circular colonies were shown. Colonies of different colors were chosen such as creamy, yellow, pale orange, pink, white and purple .15 colonies showed entire margin. 11 colonies showed convex elevation, 1 colony showed flat elevation and 3 colonies showed raised elevation. 8 colonies

showed smooth surface, 1 showed dry surface, 1 showed powdery surface and 5 showed glistening surface .All growth were observed on agar surface.

Table-26: Morphological characteristics of bacterial cells isolated from the rearing water samples of the Cultured Pond grown on different culture media (Nutrient,MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
NWS_{1a}	-	Rod	Arranged in single cell
NWS_{1b}	-	Rod	Arranged in long chain
NWS_{2a}	-	Rod	Arranged in small chain
NWS_{2b}	-	Rod	Arranged in long chain
NWS_{3a}	-	Short rod	Arranged in small chain
NWS_{3b}	-	Rod	Arranged in long chain
MWS_{1a}	-	Rod	Arranged in single and pair
MWS_{1b}	-	Rod	Arranged in single and pair
MWS_{2a}	-	Rod	Arranged in long chain
MWS_{2b}	-	Rod	Arranged in single cell
MWS_{3a}	-	Rod	Arranged in single cell
MWS_{3b}	x	x	x
SWS_{1a}	-	Short rod	Arranged in small chain
SWS_{1b}	x	x	x
SWS_{2a}	x	x	x
SWS_{2b}	-	Rod	Arranged in single and pair
SWS_{3a}	-	Rod	Arranged in long chain
SWS_{3b}	-	Rod	Arranged in single cell
$TWS_{1(a+b)}$	x	x	x
$TWS_{2(a+b)}$	x	x	x
$TWS_{3(a+b)}$	x	x	x

x = No growth, NWS= Nutrient Agar Water Sample, MWS= MacConkey Agar Water Sample, SWS= Salmonella- Shigella Agar Water Sample, TWS= Thio Sulfate Citrate Bile salts Sucrose Agar Water Sample.

According to table no 26 from the rearing water samples of the Cultured Pond -

Total strains were 24. But growths were observed in 15 strains and no growth found in 9 strains. All of them, 15 isolates were gram negative bacterial strains shown. Among those 13 were rod and 2 were short rod. Among

them 3 were arranged in single and pair, 3 were in small chain, 5 were arranged in long chain and 4 were arranged in single cell.

Table-27: Biochemical characteristics of gram negative bacterial isolated from the rearing water samples of Cultured Pond grown on different media (MacConkey, SS and TCBS*)

Stain	Gram reaction	Slant	Butt	Gas	Motility	Indole	Urease	Catalase	Oxidase	Isolates
MWS_{1a}	-	A	A	+	-	+	+	+	-	*Klebsiella*
MWS_{1b}	-	K	A	-	+	+	-	+	+	*Plesiomonas*
MWS_{2a}	-	K	A	-	+	+	+	+	+	Unidentified
MWS_{2b}	-	K	A	+	+	+	-	+	+	*Aeromonas*
MWS_{3a}	-	K	A	-	+	+	-	+	+	*Pseudomonas*
MWS_{3b}	-	K	A	+	+	-	-	+	+	*E.coli*
SWS_{1a}	-	K	A	+	+	+	-	+	+	Unidentified
SWS_{1b}	x	x	x	x	x	x	x	x	x	x
SWS_{2a}	x	x	x	x	x	x	x	x	x	x
SWS_{2b}	-	K	K	+	+	-	-	+	-	*Shigella*
SWS_{3a}	-	A	A	+	+	+	+	+	+	Unidentified
SWS_{3b}	-	K	A	+	+	-	-	+	-	*Salmonella*

x = No growth, NWS= Nutrient Agar Water Sample, MWS= MacConkey Agar Water Sample,SWS= Salmonella- Shigella Agar Water Sample, TWS= Thio Sulfate Citrate Bile salts Sucrose Agar Water Sample. K = Alkaline (red) reaction, A = Acidic (yellow) reaction, + = Positive reaction,

****No growth was observed in TCBS* agar.***

According to table no 27 from the rearing water samples of Cultured Pond

Total strains were 12 and growths were observed in 10l strains. KIA test indicated that out of 10 strains, 2 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 8 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 10 strains, 7 produced gases which were observed by formation of bubbles in the media and 3 were not produce gas. MIU test indicated that out of 10 strains, 9 strains were motile and 1 was non- motile. Iodole test were positive for 7 rods. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains 3

showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test:

All the isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 10 gram negative rods, 6 were oxidase positive and 4 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table-28: Comparative analysis of bacterial cells present in rearing water of Open Water (Lake) and Cultured Pond

Name of the bacteria	Rearing water	
	Open Water(lake)	Cultured Pond
Shigella	+	-
Salmonella	+	-
Pseudomonas	+	+
Plesiomonas	+	+
Aeromonas	+	+
Klebseilla	-	-
E.coli	+	+
Vibrio	+	-
Yersinia	-	-
Enterobacter	+	-

+= Presence, - = Absence

From the table no 28, it were observed that in Open Water (lake) *Salmonella, Shigella, E.coli,Plesiomonas,Pseudomonas, Aeromonas, Vibrio, Enterobacter,* were present and *Klebseilla, Yersinia* were absent .In rearing water sample of

Cultured Pond *E.coli,Plesiomonas,Pseudomonas, Aeromonas,* were present but *Salmonella, Shigella, Klebseilla, Yersinia, Vibrio, Enterobacter* were absent.

Table-29: Different parameters of the rearing water samples of Open Watre (Lake) and Cultured Pond

Parameters	Unit	Lake	Pond
Temperature	^{O}C	30.4	28.93
Dissolved Oxygen (DO)	ppm	5.76	7.24
Biological Oxygen demand (BOD)	ppm	35.8	1.97
P^{H}		6.92	6.94

Source: "Pond Management" a publication of GenoMar Supreme Tilapia™ Inc. Norway.

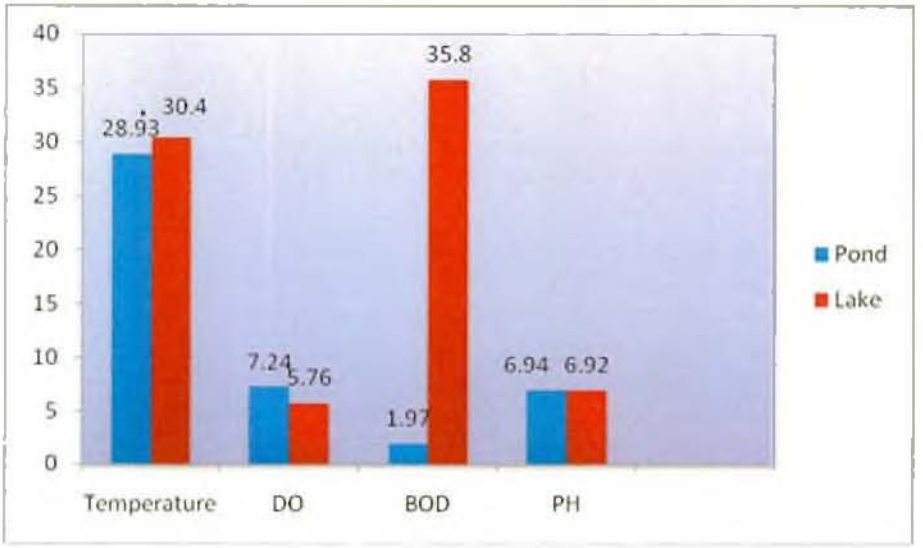


Figure 3: Comparison of Different parameters of the rearing water samples Open Water (Lake) and Cultured Pond

The figure shows the comparison between different parameters of the rearing water samples of (lake) and Cultured Pond. From the study it was observed that though there were slight difference between Open Water (lake) and Cultured Pond water in terms of temperature, p^{H} and DO level but BOD level of Open

Water (lake) were much higher than that of Cultured Pond water. The BOD of Open Water (lake) was 35.8 ppm where in case of Cultured Pond water it was 1.97ppm.

Table-30: Bacterial load in intestine and mucus of fish and rearing water grown on different culture media

Types of sample			Nutrient	MacConkey	TCBS	SS
Cultured (Pond)	Fish	Intestine	+++	+++	-	++
		Mucus	+++	+++	-	-
	Water		+++	+++	-	-
Open water (Lake)	Fish	Intestine	+++	+++	+	++
		Mucus	+++	+++	+	++
	Water		+++	+++	+	++

+++ = Growth more than 10^6 cfu/g
++ = Growth more than 10^3 cfu/g
+ = Growth more than 10^2 cfu/g
- = No growth

From the table 30 no, for the all sample growth on NA, MacConkey is higher than on SS, TCBS. In case of cultured fish samples and pond water sample no growth shows on TCBS media Incase of cultured fish samples in mucus and pond water sample no growth was observed on SS media.

Table 31: Total bacterial count of the selected fish feeds grown on Nutrient and MacConkey agar media

Sample	Nutrient Agar (cfu/g)	Mac Conkey Agar (cfu/g)
Fish Feed1	$1.75x\ 10^9$	$1.65x10^8$
Fish Feed 2	$1.1x\ 10^8$	$2.15x\ 10^8$

From the Table 31 showed that average bacterial load of fish feed 1 grown on Nutrient agar and MacConkey agar were $1.75x\ 10^9$ and $1.65x10^8$(cfu/g) , respectively where in case of fish feed 2 it were $1.1x\ 10^8$ and $2.15x\ 10^8$(cfu/g) .

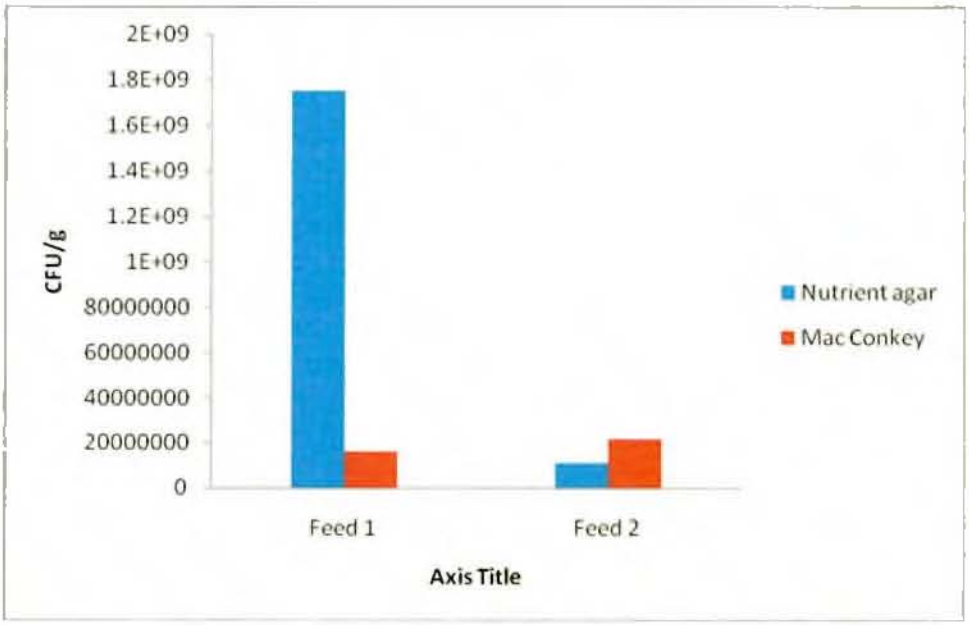


Figure 4: Total bacterial counts (cfu/ g) of the selected fish feeds grown on Nutrient and MacConkey agar media

Table 32: Composition of the selected fish feeds from locally available sources

Ingredients	Fish Feed 1*	Fish Feed 2**
Rice bran	25	20
Wheat bran	30	14
Maize	15	30
Mustard oil cake	10	15
Meat and poultry Viscera	15	15
Vitamin and mineral premix	1	1.5
Fish meal enzyme	0	0.5
Salt	4	4
Total	**100**	**100**

Powder form, ** Pellet form

The composition of the selected two fish feeds collected from the locally available sources were shown in table-32. Both the fish feeds were mainly consists of rice bran (%FF_1=25% and F_2= 20%), wheat bran (FF_1=30% and FF_2 = 14%), Maize (FF_1=15% and FF_2 = 30%) and mustard oil cake (FF_1=10% and FF_2 = 15%). The amounts of meat and poultry viscera were same (15%) in both feeds. Both fish feeds contain vitamin and mineral premixes (%FF_1=1% and FF_2= 1.5%), fish meal enzyme were only present in fish feed-2(FF_2) at 0.5% level.

Table 33: Proximate composition (% dry matter) of the selected fish feeds

Parameters	Fish Feed 1*(FF_1)	Fish Feed 2**(FF_2)
Dry matter	91.26	87.26
Protein	27.32	25.10
Fat	3.19	1.96
Crude Fibre	16.75	15.76
Ash	11.20	10.21
Carbohydrate*	32.80	34.23
Moisture	8.74	12.74

Powder form, ** Pellet form

*** Carbohydrates:** ***100- % (Moisture +Protein +Fat + Crude fibre +Ash)***

Table33 shows the proximate composition of the selected fish feeds. It was observed that fish feed-1(FF_1) and fish feed-2(FF_2) contains 87.26% and 91.26% dry matter respectively. Moisture content were higher in FF_1 (12.74%) compare to FF_2 (8.74%). The other constituents were very similar to each other such as protein 27.32%verses 25.10%,fat 3.19% verses1.96% and carbohydrate were 32.80%verses 34.23% for FF_1 and FF_2.

Table-34: Cultural characteristics of bacterial cells isolated from fish feed-1 grown on different culture media (Nutrient, MacConkey, SS and TCBS)

Strain	Colony shape	Color	Margin	Surface	Elevation	Growth on Agar
$NFFS_{1a}$	Circular	Creamy	Entire	Smooth	Raised	Surface
$NFFS_{1b}$	Circular	Yellow	Entire	Smooth	Convex	Surface
$NFFS_{1c}$	Circular	White	Entire	Smooth and glistening	Convex	Surface
$MFFS_{1a}$	Circular	Purple	Entire	Smooth and glistening	Flat	Surface
$MFFS_{1b}$	Circular	Creamy	Entire	Smooth and glistening	Flat	Surface
$MFFS_{1c}$	Circular	Pink	Entire	Smooth and glistening	Flat	Surface
$SFFS_{1a}$	Circular	Yellow	Undulate	Smooth	Raised	Surface
$SFFS_{1b}$	Circular	Black	Entire	Smooth and glistening	Flat	Surface
$SFFS_{1c}$	Circular	Pale pink	Entire	Smooth and glistening	Flat	Surface
$TFFS_{1a}$	Circular	Greenish	Entire	Smooth and glistening	Convex	Surface
$TFFS_{1b}$	Irregular	Yellowish	Undulate	Smooth & glistening	Raised	Surface
$TFFS_{1c}$	Circular	Greenish	Entire	Smooth and glistening	Convex	Surface

$NFFS_1$= Nutrient Agar Fish Feed Sample1, $MFFS_1$= MacConkey Agar Fish FeedSample 1 , $SFFS_1$= Salmonella- Shigella Agar Fish Feed Sample1, $TFFS_1$= Thio Sulfate Citrate Bile salts Sucrose Agar Fish Feed Sampl 1.

According to table No 34 from the Fish Feed -1 sample -

Total strains were 12. Growths were observed in all strains. 11 circular colonies were showed and 1 irregular colony was showed. Colonies of different colors were chosen such as creamy, yellow, pale pink, pink, white, black, greenish, yellowish and purple .10 colonies showed entire margin, and 2 colonies showed undulate margin. 4 colonies showed convex elevation, 5 colonies showed flat elevation and 3 colonies showed raised elevation. 3colonies showed smooth surface and 9 colonies showed smooth and glistening surface .All growth were observed on agar surface.

Table 35: Morphological characteristics of bacteria isolated from fish feed-1grow on different media (Nutrient, MacConkey, SS and TCBS).

Strain	Gram reaction	Shape	Arrangement
$NFFS_{1a}$	-	Rod	single cell
$NFFS_{1b}$	+	Coccus	Scattered
$NFFS_{1c}$	-	Short rod	single cell
$MFFS_{1a}$	-	Rod	Single chain
$MFFS_{1b}$	-	Rod	Single and pair
$MFFS_{1c}$	-	Rod	Long chain
$SFFS_{1a}$	-	Rod	Single and pair
$SFFS_{1b}$	-	Rod	Single and pair
$SFFS_{1c}$	-	Rod	Single and pair
$TFFS_{1a}$	-	Rod, coma	Single cell
$TFFS_{1b}$	-	Rod,Coma	Single and pair
$TFFS_{1c}$	-	Coma	Single and pair

$NFFS_1$= Nutrient Agar Fish Feed Sample1, $MFFS_1$= MacConkey Agar Fish FeedSample 1 , $SFFS_1$= Salmonella- Shigella Agar Fish Feed Sample1, $TFFS_1$= Thio Sulfate Citrate Bile salts Sucrose Agar Fish Feed Sampl 1.

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According to table no 35 from the Fish Feed -1 sample of the cultured Tilapia -

Total strains were 12. Growths were observed in 12 strains. All of them, 11isolates were gram negative bacterial strain shown. Among those 7 were rod, 1 was short rod, 1 was coma, 1 was coccus and 2 were rod, coma. Among them 6 were arranged in single and pair, 1was in single chain, 1 was arranged in long chain, 3 were arranged in single cell and 1was in scattered cell.

Table 36: Biochemical characteristics of bacterial cells isolated from fish feed-1 grown on different media (Nutrient, MacConkey, SS and TCBS

Strain	Gram Reaction	Slant	Butt	Gas	Motility	Indole	Urease	catalase	Oxidase	Isolates
$NFFS_{1a}$	-	K	A	+	-	+	+	+	+	Unidentified
$NFFS_{1b}$	+	K	A	+	-	-	-	+	-	*Staphylococcus*
$NFFS_{1c}$	-	A	A	+	+	+	-	+	-	*E.coli*
$MFFS_{1a}$	-	A	A	+	+	-	-	+	-	*E.coli*
$MFFS_{1b}$	-	A	A	+	+	-	-	+	-	*E.coli*
$MFFS_{1c}$	-	A	A	+	+	-	-	+	-	*E.coli*
$SFFS_{1a}$	-	K	A	+	+	-	-	+	-	*Salmonella*
$SFFS_{1b}$	-	K	A	-	-	-	-	+	-	*Shigilla*
$SFFS_{1c}$	-	K	A	-	-	-	-	+	-	*Shigilla*
$TFFS_{1a}$	-	K	A	-	+	+	-	+	+	*Vibrio*
$TFFS_{1b}$	-	K	A	-	+	+	-	+	+	*Vibrio*
TFF_{1c}	-	K	A	-	+	+	-	+	+	*Vibrio*

$NFFS_1$= Nutrient Agar Fish Feed Sample1, $MFFS_1$= MacConkey Agar Fish FeedSample , $SFFS_1$= Salmonella- Shigella Agar Fish Feed Sample1, $TFFS_1$= Thio Sulfate Citrate Bile salts Sucrose Agar Fish Feed Sampl 1. K = Alkaline (red) reaction, A = Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction,

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According to table no 36 from the Fish Feed -1 sample -

Total strains were 12 and growths were observed in all strains. KIA test indicated that out of 12 strains, 4 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 8 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 12 strains, 5 produced gas which was observed by formation of bubbles in the media.

MIU test indicated that out of 12 strains, 8 strains were motile and 4 were non-motile. Iodole test were positive for 6 rods. These were indicating by red color of

the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains 2 showed urease activity. Urease activity was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test:

11 isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 11 gram negative rods, 2 were oxidase positive and 10 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table-37: Cultural characteristics of bacterial cells isolated from fish feed-2 grown on different culture media (Nutrient, MacConkey, SS and TCBS).

Strain	Colony shape	Color	Margin	Surface	Elevation	Growth on Agar
$NFFS_{2a}$	Irregular	Creamy	Undulate	Smooth	Flat	Surface
$NFFS_{2b}$	Circular	White	Entire	Dry & powdery	Convex	Surface
$NFFS_{2c}$	Circular	White	Entire	Smooth & glistening	Flat	Surface
$MFFS_{2a}$	Circular	Purple	Entire	Smooth and glistening	Flat	Surface
$MFFS_{2b}$	Circular	Creamy	Entire	Smooth and glistening	Flat	Surface
$MFFS_{2c}$	Circular	Pink	Entire	Smooth and glistening	Flat	Surface
$SFFS_{2a}$	Circular	Pale pink	Entire	Smooth and glistening	Flat	Surface
$SFFS_{2b}$	Circular	Black	Entire	Smooth and glistening	Flat	Surface
$SFFS_{2c}$	Circular	Creamy	Entire	Smooth and glistening	Flat	Surface
$TFFS_{2a}$	Punctiform	Yellow	Entire	Smooth and glistening	Convex	Surface
TFF_{Sb}	Punctiform	Yellow	Entire	Smooth and glistening	Flat	Surface
$TFFS_{2c}$	Irregular	Yellow	Undulate	Smooth and glistening	Raised	Surface

$NFFS_2$= Nutrient Agar Fish Feed Sample 2, $MFFS_2$= MacConkey Agar Fish FeedSample 2, SFFS2= Salmonella- Shigella Agar Fish Feed Sample2, $TFFS_2$= Thio Sulfate Citrate Bile salts Sucrose Agar Fish Feed Sampl 2.

According to table No 37 from the Fish Feed -2 samples -

Total strains were 12. Growths were observed in all strains. 8 circular colonies were shown. 2 colonies showed irregular in shape and 2 colonies showed punctiform in shape. Colonies of different colors were chosen such as creamy, yellow, pale pink, white purple and black .10colonies showed entire margin, and 2colonies showed undulate margin. 2 colonies showed convex elevation, 9 colonies showed flat elevation and 1 colony showed raised elevation. 24 colonies

showed smooth surface, 1 showed rough surface and 7colonies showed glistening surface .All growth were observed on agar surface.

Table 38: Morphological characteristics of bacteria isolated from fish feed-2 grown on different media (Nutrient, MacConkey, SS and TCBS)

Strain	Gram reaction	Shape	Arrangement
$NFFS_{2a}$	-	Rod	Long chain
$NFFS_{2b}$	-	Rod	Long chain
$NFFS_{2c}$	-	Coccus	Single and pair
$MFFS_{2a}$	-	Rod	Single chain
$MFFS_{2b}$	-	Rod	Single and pair
$MFFS_{2c}$	-	Rod	Single and pair
$SFFS_{2a}$	-	Short rod	Small chain
$SFFS_{2b}$	-	Rod	Single and pair
$SFFS_{2c}$	-	Rod	Single and pair
$TFFS_{2a}$	-	Rod, coma	Single chain
TFF_{Sb}	-	Coma,	Single and pair
$TFFS_{2c}$	-	Coma	Single and pair

$NFFS_2$= Nutrient Agar Fish Feed Sample 2, $MFFS_2$= MacConkey Agar Fish FeedSample 2,$SFFS_2$= Salmonella- Shigella Agar Fish Feed Sample2, $TFFS_2$= Thio Sulfate Citrate Bile salts Sucrose Agar Fish Feed Sampl 2.

According to table no 38 from the Fish Feed -2 samples -

Total strains were 12. Growths were observed in all strains. All of them were gram negative bacterial strains shown but 1 was gram positive. Among those 7 were rod, 1 was short rod, 1 was rod, coma, 1 was coccus and 2 were coma. Among them 7 were arranged in single and pair, 1 was in small chain, 2 were arranged in long chain and 2 were arranged in single chain.

Table 39: Biochemical characteristics of bacterial cells isolated from fish feed-2 grown on different media (Nutrient, MacConky, SS and TCBS)

Strain	Gram Reaction	Slant	Butt	Gas	Motility	Indole	Urea	catalase	Oxidase	Isolates
$NFFS_{2a}$	-	K	A	++	-	+	+	+	+	Unidentified
$NFFS_{2b}$	-									
$NFFS_{2c}$	+	K	A	+	-	-	-	+	-	*Staphylococcus*
$MFFS_{2a}$	-	A	A	+	+	+	-	+	-	*E.coli*
$MFFS_{2b}$	-	A	A	+	+	+	-	+	-	*E.coli*
$MFFS_{2c}$	-	A	A	+	+	+	-	+	-	*E.coli*
$SFFS_{2a}$	-	K	A	+	+	-	-	+	-	*Salmonella*
$SFFS_{2b}$	-	K	A	-	-	-	-	+	-	*Shigilla*
$SFFS_{2c}$	-	K	A	-	-	-	-	+	-	*Shigilla*
$TFFS_{2a}$	-	K	A	-	+	+	-	+	+	*Vibrio*
TFF_{Sb}	-	K	A	-	+	+	-	+	+	*Vibrio*
$TFFS_{2c}$	-	K	A	-	+	+	-	+	+	*Vibrio*

$NFFS_2$= Nutrient Agar Fish Feed Sample 2, $MFFS_2$= MacConkey Agar Fish FeedSample 2, SFFS2= Salmonella- Shigella Agar Fish Feed Sample2, $TFFS_2$= Thio Sulfate Citrate Bile salts Sucrose Agar Fish Feed Sampl 2. K = Alkaline (Red) reaction, A = Acidic (yellow) reaction, + = Positive reaction, - = Negative reaction,

According to table no 39 from the Fish Feed -2 samples -

Total strains were 12 and growths were observed in all strains. KIA test indicated that out of 12 strains, 4 strains gave positive results for both glucose and lactose fermentation. The strains produced acid from both glucose and lactose so that both the butt and slant turned yellow. 8 strains fermented glucose but not lactose. So the butt turned into yellow but the slant remained red. Out of 12 strains, 6 produced gas which was observed by formation of bubbles in the media.

MIU test indicated that out of 12 strains, 9 strains were motile and 3 were non-motile. Iodole test were positive for 6 rods. These were indicating by red color of the strips which were impregnated with Kovac's reagent and were attached to the top of the tube. Among the strains 2 showed urease activity. Urease activity

was indicating by a red color in the MIU medium. The red color occurs when urea is hydrolyzed causing an alkaline p^H.

Catalase Test:

From 12 isolates, 2 isolates having rod shape were rod catalase positive. Formation of the bubbles after addition of H_2O_2 solution on the smears of fresh culture indicated the presence of catalase.

Oxidase Test:

While carrying out the oxidase test, in the filter paper 2-3 drops of oxidase reagent was placed and then the fresh culture was smeared. Out of 12 gram negative rods, 11were oxidase positive and 1 were negative. Positive reaction was indicated by the appearance of the dark purple color on the filter paper within 10 seconds.

Table-40: Comparative analysis of bacteria present in fish feed 1 and fish feed 2

Names of the bacteria	Fish Feed 1	Fish Feed 2
Salmonella	+	+
Shigella	+	+
E.coli	+	+
Vibrio	+	+
Staphylococcus	+	+

+= Presence,

From the table no 40, it was observed that in fish feed 1 *Salmonella, Shigella, E.coli, Vibrio Spp.* and *Staphylococcus* were present while in case of fish feed 2 all the above mentioned bacteria were present .

Chepter-5:

- Discussion

5. Discussion

In the present study the effect of water quality on the micro flora of Tilapia (*Oreochromis niloticus*) reared in two different conditions Open Water (Lake) and cultured, was studied. The major objective of the present study was to compare the total viable bacterial count (TVBC) quantitatively and qualitatively, present in the mucus and intestine of both groups fish of as well as rearing water. Emphasis was given on whether there is an association of pathogenic bacteria with the fish and rearing water or not. Two groups of fish each consisted of five fish samples were collected from Gajipur Farm (cultured fish) and Dhanmondi lake (wild fish) during the month of August to November' 2006. Wild fish did not receive any special feed but cultured fish were reared with selected fish feed. In the present study the quality of the fish feed and there microbial count was also observed. Two different types of fish feed samples powder form (FF_1) and pellet form (FF_2), were collected from locally available sources at Gagipure.

Isolation of bacteria was made on Nutrient, MacConky, SS and TCBS agar. MacConky, SS and TCBS agar were employed to facilitate the growth and isolation of gram negative bacteria, *Salmonella* and *Shigella spp.* *Vibrio spp.* respectively. Nutrient agar was used to isolate non fastidious organisms.The bacterial isolates were characterized and identified by their cultural, morphological and biochemical characteristics.

From the study it was observed that The average viable bacterial count in the mucus of Open Water type Tilapia fish on Nutrient and MacConkey agar were 9.9×10^5 and 1.08×10^5 (cfu/g) whereas in the intestine the average bacterial count (cfu/g) were 1.85×10^9 and 1.73×10^8 (cfu/g) respectively. Total viable bacterial count in the mucus of Cultured Tilapia fish on Nutrient and MacConkey agar were 1.29×10^5 and 7.03×10^4 (cfu/g) while in the intestine the average viable bacterial counts were 4.96×10^6 and 3.60×10^6 (cfu/g) respectively. Therefore it was observed that the Culture Tilapia fishes carried lower bacterial content than Open Water type Tilapia in both intestine and

mucus on Nutrient and MacConkey agar media. Similar study conducted by Ahmed H. Al-Habra & Naim Uddin showed that Total viable count of bacteria in Open Water (Lake) tilapia was higher than in cultured tilapia. The bacterial count found in our study was far higher than the acceptable level.

By considering the cultural, morphological and bio chemical characteristics of the isolated strains it was observed that in mucus and intestine of Open Water fish *Shigella, Salmonella, Pseudomonas, Plesiomonas, Aeromonas, Klebseilla, E.coli, Vibrio, Yersinia, Enterobacter* were present while in case of mucus of cultured fish *Vibrio, Yersinia, Enterobacter* were absent and in intestine of cultured fish *Vibrio, Yersinia, Enterobacter, , Klebseilla* were absent. So it is revealed that the pathogenic bacteria *Salmonella, Shigella and Vibrio* were present only in the Open water type tilapia which indicates better microbial management for cultured fish. In another study Ahmed H. Al-Habra & Naim Uddin showed association of same types of bacteria in the intestine of hybrid tilapia (*Oreochromis niloticus*).

The present study also showed that total viable bacterial counts in the rearing water samples of Open Water Tilapia (lake) on Nutrient and MacConkey agar were 1.75×10^5 (cfu/ml) and 0.96×10^5 (cfu/ml) where in the rearing water samples of the pond (cultured) average bacterial counts were 1.45×10^4(cfu/ml) and 1.10×10^4 (cfu/ml) respectively. In Bangladesh, Banu *et al.* (2001), investigated the bacterial load in pond water. They observed that the mean bacterial load in surface water varied from 1.39×10^5 (July 94) to 3.11×10^7 CFU/ml (September 93), while that of bottom water ranged from 1.0×10^6 (May 94) to 5.90×10^7 CFU/ml (October 93). Romanenko (1971) reported that the bacterial number in reservoir water was $1.43\text{-}0.18 \times 10^6$ ml of water. Tewary and Mishra (1985) reported that fresh water bacteria varied from 1 to 3.00×10^3 CFU/ml in the lake water. Araki and Kitamikadi (1978) pointed out that the population density of bacteria ranged from 0.0 to 1.8×10^5 cell/ml of water in some river and pond water of Japan. As we did not filter the water sample the count was around 10^5 to 10^6 cfu/g which may be far more greater if concentrate

by filtration. The higher number of microorganisms in lake water reflected by higher number of microorganisms in the fish body.

From the study it was observed that in Open Water (lake) *Salmonella, Shigella, E.coli,Plesiomonas,Pseudomonas, Aeromonas, Vibrio, Enterobacter,* were present and *Klebseilla, Yersinia* were absent. In rearing water sample of Cultured Pond *E.coli,Plesiomonas,Pseudomonas, Aeromonas,* were present but *Salmonella, Shigella, Klebseilla, Yersinia, Vibrio, Enterobacter* were not present. Another study was conducted for the determination of the microbial quality of pond water and prevalence of bacterial pathogens by using the disk diffusion method.

In pond water BOD ,DO, P^H, and temperature were measured and found that the average biological oxygen demand (BOD) was 1.97 ppm, dissolved oxygen (DO) was 7.24 ppm ,P^H was 6.94 and temperature was 28.93 ^{0}C.In lake water the average biological oxygen demand (BOD) was35.8 ppm, dissolved oxygen (DO) was 5.76 ppm ,P^H was 6.92 and temperature was 30.4 ^{0}C. From the study it was observed that though there was slight difference between Open Water (lake) and Cultured Pond water in terms of temperature, p^H and DO level but BOD level of Open Water (lake) was much higher than that of Cultured Pond water. All the measurements of pond water showed an ideal condition for cultured fish than lake water for wild fish. In the experiment the total viable bacterial count of cultured fish was very low and the mucus had a lower viable count of bacteria than intestine.BOD level of pond water is very good. There will not be much organic waste present in the rearing water of pond. BOD level of lake water is very law. The water is considered much polluted with organic waste. Generally, as BOD levels increase there is a corresponding decrease in DO levels. According to "Pond Management" a publication of GenoMar Supreme Tilapia, Norway the safe range of temperature, DO, BOD and p^H in pond water was 28-32, >4,<2 and 6.5-8.8 respectively.

The average bacterial load of fish feed 1 grown on Nutrient agar and MacConkey agar was $1.75x\ 10^9$ and $1.65x10^8$ respectively where in case of fish

feed 2 it was 1.1x 10^8 and 2.15x 10^8. The composition of the selected two fish feeds collected from the locally available sources showed that doth the fish feeds are mainly consists of rice bran (%FF_1=25% and F_2= 20%), wheat bran (FF_1=30% and FF_2 = 14%), Maize (FF_1=15% and FF_2 = 30%) and mustard oil cake (FF_1=10% and FF_2 = 15%). The amount of meat and poultry viscera was same (15%) in both feeds. Both fish feeds contain vitamin and mineral premixes (%FF_1=1% and FF_2= 1.5%), fish meal enzyme was only present in fish feed-2(FF_2) at 0.5% level. From the proximate composition of the selected fish feeds it was observed that fish feed-1(FF_1) & fish feed-2(FF_2) contains 87.26% and 91.26% dry matter respectively. Moisture content was higher in FF_1 (12.74%) compare to FF_2 (8.74%). The other constituents were very similar to each other such as protein 27.32%verses 25.10%, fat 3.19% verses1.96% and NFE (Nitrogen Free Extract) was 32.80%verses 34.23% for FF_1 and FF_2. From the study it was observed that both in fish feed 1and fish feed 2 *Salmonella, Shigella, E.coli, Vibrio Spp.* and *Staphylococcus* were present.

It is also likely to say that the toxic product from industries and house complex are poured in to the lake water though sewerage line where Tilapia fish are cultured. This waste product can infect the fishes and by ingestion of those fish carring deadly pathogenic bacteria may deteriorate the health of the consumers or even lead to death. The news media are trying their level best to highlight all those events occurring in our daily life for creating awareness among the people. Public health department already working vigorously to give emphasis on it. Various study from different parts of the world have demonstrated that may enteric organisms are associated with fish and they can initiate various fish born diseases in man. In Bangladesh very limited study has been done on isolation of bacterial pathogen from fish. Moreover, it is well known that prevalence and variety of bacteria are directly related to temperature and water quality of the rearing environment. Therefore the aim of the present study is isolate and identify the potential Gram- negative human pathogenic bacteria from mucus and intestine of Tilapia (*Oreochromis niloticus*) reared in two different farms and also to determine the water quality of the rearing farms as the hygienic

condition of the water effect the quantitative and qualitative aspects of the fish bacterial flora.

Chepter-6:

- Conclusion

CONCLUSION

1. Total viable bacterial count was higher in the Open Water (lake) Tilapia than in the cultured Tilapia .
2. The bacterial load found in the Open Water (lake) Tilapia was far greater than the acceptable (safe) level.
3. Frequently encountered bacteria in the samples of both the Open Water (lake) and Cultured fish as well as in the rearing water and fish feeds (FF_1 and FF_2) were *Aeromonas, Plesiomonas, Escherichia coli, Pseudomonas, Salmonella, Shigella,* and *Klebsiella*. The bacteria were predominantly Gram negative rods 91% .
4. Pathogenic bacteria such as *Vibrio* spp, *Salmonella, Shigella were* found in the intestine and mucus of the Open Water (lake) Tilapia, rearing water of lake (Dhanmondi) and Fish Feeds (FF_1 and FF_2). But they were absent in the Cultured fish samples as well as in pond water.
5. Basic parameters such as BOD,DO, P^H and temperature of the Open water (Dhanmondi lake, Dhaka) do not meet up to the normal range, but the water quality of cultured pond was far better.
5. TVBC was higher in the rearing water of Open Water (lake) than in the pond.
7. Fish feed (FF1) was higher in moisture content than in the Fish feed (FF2). Because of higher moisture content more bacterial growth was observed.

Chepter-7:

- Recommendations

7. Recommendation

To reduce health hazards such as food borne disease(Diarrhoea, Cholera etc) initiates the cultivation of fish maintaining healthy aquatic life to prevent contamination of food (Fish).

To research into fish feed quality could benefit all hatchery and aquaculture operations and assist in the interpretation of the effects of populationon open water fish.

To research on fish nutrition should be undertaken in order to develop cheap but good quality feed. Identification of potential future constraints to sectoral growth is an equally important area of research.

It was a small scale study . It has limitations. So, to reach a concrete decision further large scale scientific research should be needed.

Chepter-8:

- Reference

8.Reference

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