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ISOLATION AND CHARACTERIZATION OF HEAVY METAL TOLERANT BACTERIA FROM EFFULENT WATER COLLECTED IN THANDALAM LAKE RANIPET

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Abstract: The present study document the isolation and characterization of Heavy Metal tolerant Bacteria collected from effulent water, Thandalam Lake Ranipet. The bacterial Isolates were biochemically analyzed for the activities of Oxidase, Catalase, MR- VP test, Motility, Indole test. The physicochemical parameters were also analyzed using standard procedures. The results clearly indicate that the after incubation green metallic sheen colonies are formed in EMB agar and no growth in both Macckonkey and cetrimide agar.

Keywords: Oxidase, Catalase, MR- VP test

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INTRODUCTION

Water pollution is a major global problem which requires ongoing evaluation and revision of Water resource policy at all levels. It has been suggested that it is the leading worldwide cause of death and diseases, and that it accounts for the death of more then 14,000 people daily. (west., 2006).

Water pollution is the contamination of water bodies. (Eg. Lakes, river, ocean, aquifer and ground water). Water pollution occurs when pollutants are discharge directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Sewage is the used water of a community and can include domestic wastewater and industrial wastewater. Combined server systems will include storm water such as road runoff which carries oils, satts, metals and asbestos. Infections from exposure to waterborne disease organisms may be subclinical or may appear as actual disease in wastewater workers. Treatment personnel have reported nausea, vomiting, indigestion, diarrhea, and flu-like complaints. (Nellie., 1997).

Global pollution is increasing, due to the variations in natural and anthropogenenic activities leading to contamination of various terrestrial and aquatic ecosystem with heavy metals, inorganic and organic compounds and radionuclide. (Faryal., 2003).

INDUSTRIALWASTEWATER:

Some industrial facilities generate ordinary domestic sewage that can be treated by municipal facilities. Industries that generate waste water high concentration of conventional pollutants (e.g oil an grease), toxic pollutants (e.g heavy metals, volatile organic compounds) or other nonconventional pollutants such as ammonia, need specialized treatment systems. Some of these facilities can install a pre-treatment system to remove the toxic components, and then send the partially treated wastewater to the municipal system. Industries generate large volumes of wastewater typically operate their own complete on-site treatment system. Some industries have been successful at redesigning their manufacturing processes to reduce or eliminate pollutants, through a process called pollution prevention.(Faryal., 2003).

The amounts of heavy metals and synthesized organic compounds generated by industrial activities have increased, and some 10,000 new organic compounds are added each year. Many of these compounds are now found in the waste water take place in manufacturing, changes also occur in the compounds from most municipalities and communities. As technological changes discharged and the resulting wastewater characteristics. Numerous compounds generated from industrial processes are difficult and costly to treat by conventional waste water treatment processes. Therefore, effective industrial pretreatment becomes an essential pan of overall water quality management program. Enforcement of an industrial pretreatment

program is a daunting task, and some of lheregulatedpollutants still escape to the municipal wastewater collection system and must be treated. (Polyanskiy.,1986).

Pollutants in watercourses can be of several origins and types, but some of the worst pollutants are those that reduce the available oxygen content. This loss can have major adverse effects on natural ecosystems if the oxygen content falls below the level necessary to support aquatic life. Chemical oxygen demand (COD), biological oxygen demand (BOD) and total organic carbon (TOC) are three main indexes used to assess this organic pollution in aqueous systems. While the TOC analysis is valid for soluble organic compounds and the BOD reflects the biodegradable part of the pollutants, the COD analysis represents the total pollution load of most wastewater discharges. As a pollution monitoring parameter, COD has the advantage of speed and simplicity over BOD, and requires less equipment compared to TOC determinations. The COD procedure is verytime-consuming, and it demands high quality personnel and expensive or poisonous reagents. In conventional COD procedures, a known amount of oxidant is added to a sample and the mixture is boiled. After the oxidation has proceeded for a given period of time, the initial concentration of organic species can be calculated by determining the amount of oxidizing agent remain in. In the case of the dichromate method. The chemical oxygen demand, represents the oxygen required for complete chemical oxidation of a sample. The theoretical COD value of samples is related to the determination of consumed oxygen concentration in samples under chemical oxidation of the organic compounds.(Chang Kim., 2001).

The determination of biochemical oxygen demand(BOD) is an empirical test in which standardisedlaboratory procedures are used to determine the relative oxygen requirements of wastewater, effluents and polluted waters. The BOD values indicate the amount of biodegradable organic material (carbonaceous demand)and the oxygen used to oxidize inorganic material such as sulphides and ferrous iron. It also may measure the oxygen used to oxidise reduced forms of nitrogen(nitrogenous demand) unless their oxidation is prevented by an inhibitor. The BOD test has its widest application in measuring waste loading to treatment plants and in evaluating the BOD removal efficiency of such treatment systems.BOD has been determined conventionally by taking a sample of water, aerating it well, placing it in a sealed bottle, incubating for a standard period of time at 201 °C in the dark, and determining the oxygen consumption in the water at the end of incubation. According to the American standard, the incubation time is 5 days and the BOD values based on this standard are called BOD_5 for short, whereas the incubation time is 7 days in the Swedish standard and the abbreviation is BOD_7. (Liu and Mattiasson., 2002).

BIOREMEDIATION:

Environment biotechnology strategies must address and solve in a long term perspective the formidable environment problems now facing the world, such as soil contamination with pesticides, metals or hydrocarbons, disposal of animal manures, treatment of reusable producs and energy from wastes. (DorinaDinu., 2011).

Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is one of the major problems facing the industrialized world today. Bioremediation, the use of microorganisms or microbial process to degrade environmental conaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up ground water soils, lagoons, sludges and process-waste streams. (Boopathy, 2000).

Bioremediation technologies are today well established for the cleanup of chemically contaminated land, and many technologies are applied commercially in large scale. The first bioremediation technologies that excavated soil in contrast to insitu technologies which aim at treatment without excavation and often is taking care of both groundwater and soil pollution. (Jorgensen., 1991).

Bioremediation is a rapidly developing fields of environment restoration, utilizing natural microbial activity to reduce the concentration and/or toxicity of various chemicals substances such as petroleum products, aliphatic aromatic hydrocarbons including poly aromatic hydrocarbons and polychlorinated biphenyl, industrial solvents phenols, benzene, acetone etc, battery liquid, pesticides and metals(arsenic, chromium, selenium, etc). (Korda., 1997).

Bioremediation, a non-destructive, cost- and treatment-effective and sometimes logistically favorable cleanup technology, attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions. (Margesin., 2001).

In situ bioremediation is a technique that can be used to reduce the spread of metal contaminants

by applying biological treatment to hazardous chemicals in soil and groundwater. In situ bioremediation has the ability to transform contaminants to less toxic compounds, making this a promising environmental cleanup technique. (Adeniji., 2004).

MECHANISMS FOR METAL REMEDIATION:

Industrial has contaminated our soil, sediment, and water sources with hazardous materials. Metal waste is often a result of industrial activities, such as mining, refining, and electroplating.

Mercury, arsenic, lead, and chromium are often prevalent at highly contaminated sites. This fact holds significant challenges for industries because these metals are difficult to remove. Therefore researchers and industries are researching metals that undergo methylation, complexation, or changes in valence state. These are noteworthy processes because they aid with the mobility and bioavailability of metals (NABIR, 2003). There is a large interest in microorganisms that can facilitate with the transformation and the removal of the metal contaminant. Remediation of metals often involves five general approaches: isolation, immobilization, mobilization, physical separation, and extraction. Immobilization and mobilization involve bioremediation processes. Industries use a combination of more than one approach to properly treat metal-contaminated sites. (Adeniji., 2004).

HEAVY METALS:

Metal pollution is global concern. The level of metals in all environments, including air, water, and soil, are increasing in some cases to toxic level, with contributions from a wide variety of industrial sources. Heavy metals are roughly defined as elements having a density over 6 g/cm^3. Among these elements, Co, Cu, Mn, Ni, Se and Zn are important in small amounts. Some metals, such as Cu, Fe, and Zn are essential at low concentration and are toxic at higher levels. The main causes of water pollution are household wastes (As, Cr, Cu, Mn, and Ni), coal fed power stations (As, Hg and Se), iron and steel production (Cr, Mo, Zn and Sb) and metal smelters (Cd, Ni, Sb, and Se) of these metals, 25% are thought to enter rivers and lakes, and their surrounding soils are also heavily polluted by these metals.(Faryal.,2003).

Metals in the environment can be divided into two classes, bioavailable soluble, nonsorbed and mobile and non- bioavailable precipitated, complexed, sorbed and non- mobile. It is the bioavailable metal concentration that taken up and is thus toxic to biological system. Some metals like Hg and Pb are highly toxic, but many other metals are also of concern, including As, Br, B, Cd, Cr, Cu, Ni, Mn, Se,Ti and Zn. Toxicity to an organisms can be defined as the inherent potential or capacity of a material to cause adverse effects on living organisms and depends upon the bioavailability of the toxicant.(Faryal., 2003).

Metal contaminants are commonly found in soils, sediments, and water. Metal pollutants can be produced through industrial processes such as mining, refining, and electroplating. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, and complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. Certain threshold concentrations, metals can become toxic to many species. Fortunately, microorganisms can affect the reactivity and mobility of metals.

Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination. *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Citrobacteia*, *Klebsilla*, and *Rhodococcus*are organisms that are commonly used in bioremediation mechanisms. (Adeniji., 2004).

The metal contaminants pose adverse health effects to those who live neare these polluted setes. Metal waste is commonly found in soil, sediments, and water. Breathing, eating, drinking and skin contact are all possible exposure routes for metal contaminants, metal such as mercury, lead and arsenic, potentially can be toxic to the kidneys, decrease mental capabilities, and cause weakness, headaches, abdominal cramps, diarrhea, and anemia. Chronic exposure to these pollutants can cause permanant kidney and brain damage. (Adeniji., 2004).

Metal have played a pivotal role in the development of human civilization. Toxic metals are common contaminants of natural waters and may adversely affect potentially important biodegradation processes occurring in the environment. Sources of these pollutants may include leachates from hazardous waste sites, discharges from industrial plants, and effluents from wastewater treatment plants. Chemical wastes at land disposal sites may contain toxic metals in particularly high concentrations. (william A. Said., 1991).

heavy metals are difficult to remove from the environment. Heavy metals are recognized to be powerful inhibitors of biodegradation activities. These metals cannot be degraded, and are ultimately indestructible. The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes. These heavy metals such as copper, cadmium, lead, zinc, nickel, mercury and chromium when accumulated in soils, water bodies they can also be present in concentrations toxic to plants, animals, humans and aquatic life. Each heavy metal has uniquebiofunctions or biotoxicities. For example, copper can enhance microbial growth at low concentrations but repress growth at high concentrations and cadmium has high toxicity at low concentrations . The presences of nonbiodegradableheavy metals in such effluents are responsible for their persistence in the food chain. Microbes play massive role in the bio-geochemical cycling of toxic heavy metals and also in cleaning up or remediating metal-contaminated environments. Microorganisms have acquired a variety of mechanisms for adaptation to the presence of toxic heavy metals.(Selvi. ,2012).

Chromium (Cr):beneficial only as an exception. Chromium mainly occurs as Cr(VI) in the divalentoxyanion chromate and as Cr(III), the trivalent cation. Reduction/oxidation reactions between the two states are thermodynamically possible under physiological conditions, thus chromate and Cr3+ are both biologically important ions. Chromate is more toxic than Cr3+, so beneficial functions of chromium can only be performed by Cr3+. In man, the chromium cation binds to a low-molecular-mass binding substance, a small polypeptide, at a ratio of 4

Cr/peptide. The resulting Cr-containing peptide is able to activate specifically the insulin receptor tyrosine kinase. These new findings explain why chromium starvation in man leads to reduced glucose tolerance with a physiological condition similar to diabetes. Chromate, on the other hand, is toxic, carcinogenic and allergenic (mason's allergy) to man.(Nies., 1999) .

Nickel (Ni): used only for a few important reactions. Free nickel occurs mostly in the Ni2+ cationic form; Ni3+ is even more unstable than Co3+. Nickel toxicity is comparable to that of cobalt, but its toxic effect on man is better documented. Nickel allergy (contact dermatitis), especially to cheap jewellery, is very common; up to 20% of the population in industrially developed countries have positive results in epicutaneous testing. Nickel has an important function in the pathogenicity of Helicobacter pylori, a gram-negative bacterium causing gastritis and peptic ulcer disease in humans. For the colonization of the gastric mucosa, H. pylori needs to produce urease to deal with the acidic environment by producing ammonia from urea. Urease production and function depend on the avail- ability of nickel.(Nies., 1999) .

Copper (Cu): a sword with two edges. The electrochemical potential of Cu2+/Cu+ is268 mV, well within the physiological range. Copper easily interacts with radicals, best with molecular oxygen. Its radical character makes copper very toxic, and many organisms are more sensitive to copper than *E.coli*. Copper toxicity is based on the production of hydroperoxideradicals and on interaction with the cell membrane. Every person in the world may have a contact with copper in coins daily;however, one has to digest about 275 coins for a lethaleeffct, as has been shown in the caseof a mentally disturbed individual. This person diedfrom copper intoxication following a massive ingestion of coins.(Nies., 1999) .

Cadmium (Cd), the best-known toxic heavy metal.The solubility product of CdS is $1.4*10^{-29}$ but $2.91*10^{-25}$ for ZnS. Thus, cadmium is more toxic than zinc.Although a tremendous amount of work has been done, especially on cadmium toxicity in microorganisms, no defined mechanisms of action have been highlighted. The effcts may be summed up under the generalheadings ``thiol-binding and protein denaturation'', ``interaction with calcium metabolism and membranedamage'' and ``interaction with zinc metabolism'', orloss of a protective function. Only in rare cases has animportant single mechanism been found. Mutation of dsbA, encoding a product required for disulfide formation, leads to cadmium sensitivity in E. coli. Thus, DsbA is a target for cadmium in theperiplasm of gram-negative bacteria. The influence of the additional proteins induced under cadmium stress in E. coli is not understood. On the molecular level, cadmium uptake is barely understood .In Ralstonia sp. CH34, and maybe also in S. cerevisiae, cadmium is accumulated by the magnesium system(s). In other bacteria, cadmium enters the cell by some manganese uptake system. In plants, cadmium is taken up by the calcium uptake system. (Nies., 1999) .

Lead (Pb) is not as bad as its reputation. Lead is no transition element, but belongs to the elementgroup IVa, C, Si, Ge, Sn, Pb. In sea water, it is evenmore rare than mercury. Owing to its lowsolubility (lead phosphate especially is insoluble, with asolubility product of 10)54) its biologically available concentration is low. Thus, lead is not extraordinarilytoxic for microorganisms.Lead has been used in large amounts for 2500 years, recently as a fuel additive, althoughthe toxicity of lead for animals and man has been wellknown for a long time . Lead acts on thecentral nervous system, on blood pressure and on re- production. In rural Albania, repair of abroken mill stone with lead and the resulting contamination of the our recently led to the death of twopeople.Lead-tolerant bacteria have been isolated, and precipitation of lead phosphate within the cells of these bacteria has been reported. In *Ralstonia sp.* CH34 it has been shown that resistance tolead is mediated by a P-type ATPase. Moreover, theCadA P-type ATPase is also able to transport Pb2+. Thus, lead resistance may also bebased predominantly on metal ion efflux.(Nies., 1999).

Zinc(zn) no life without zinc. Zinc occurs exclusively as the divalent cation Zn2+.With its completely filled d orbitals, the zinc cation is notable to undergo redox changes under biological conditions. It is used to complex polypeptide chains, for example, when redox reactions are not desired, and, as aLewis base, mainly to activate water .Zinc is a component in such a variety of enzymes andDNA-binding proteins, such as zinc-finger proteinswhich also exist in bacteria, that lifeseems not to be possible without this redox-inactiveformer of tight complexes.The toxicity of zinc to E. coli is similar to the toxicityof copper, nickel and cobalt. Zinc toxicity inman may be based on zinc-induced copper deficiency. Zinc is less toxic than copper, in amentally disturbed human, 461 zinc-containing coinswere required for a lethal effect .Zinc may be complexed by various cellular components, and is transported by members ofa variety of protein families.(Nies., 1999).

LEAD:

Lead is a chemical element in the carbon group with symbol Pb . Lead is a soft and malleable metal, which is regarded as a heavy metal and poor metal. Metallic lead has a bluish-white color after being freshly cut, but it soon tarnishes to a dull grayish color when exposed to air. Lead has a shiny chrome-silver luster when it is melted into a liquid. Lead, at certain contact degrees, is a poisonous substance to animals, including humans. It damages the nervous system and causes brain disorders. Excessive lead also causes blood disorders in mammals. Like the element mercury, another heavy metal, lead is a neurotoxin that accumulates both in soft tissues and the bones.Lead is a bright and silvery metal with a very slight shade of blue in a dry atmosphere. Upon contact with air, it begins to tarnish by forming a complex mixture of compounds depending on the conditions. The color of the compounds can vary. The tarnish layer can contain significant amounts of carbonates and hydroxycarbonates. It has a few

characteristic properties: high density, softness, ductility and malleability, poor electrical conductivity compared to other metals, high resistance to corrosion, and ability to react with organic chemicals. *(Polyanskiy.,1986).*

Lead (Pb)is not as bad as its reputation. Lead is no transition element, but belongs to the elementgroup IVa, C, Si, Ge, Sn, Pb. In sea water, it is evenmore rare than mercury. Owing to its lowsolubility (lead phosphate especially is insoluble, with asolubility product of 10)54) its biologically available concentration is low. Thus, lead is not extraordinarilytoxic for microorganisms.Lead has been used in large amounts for 2500 years, recently as a fuel additive, althoughthe toxicity of lead for animals and man has been wellknown for a long time . Lead acts on thecentral nervous system, on blood pressure and on re- production. In rural Albania, repair of abroken mill stone with lead and the resulting contamination of the our recently led to the death of twopeople.Lead-tolerant bacteria have been isolated, and precipitation of lead phosphate within the cells of these bacteria has been reported. In *Ralstonia sp.* CH34 it has been shown that resistance tolead is mediated by a P-type ATPase. Moreover, theCadA P-type ATPase is also able to transport Pb2+. Thus, lead resistance may also bebased predominantly on metal ion efflux.(Nies., 1999).

HEALTH AND TOXICOLOGY:

Lead is commonly found in industrial settings and lead exposure has the tendency to cause adverse health effects. The adverse health effects induced by lead exposure are dependent on two important components: dose (how much of a contaminant) and duration (how long there has been contact with the contaminant). Exposure to lead can occur from eating anddrinking contaminated water or breathing in high levels of lead. Children areexposed to lead through eating lead based paint chips or through skin absorption while playing inlead contaminated soil. This hazardous material enters the air we breathe, the water we drink,and the plants and animals we consume.(Adeniji., 2004).

Once lead has entered the blood stream, it flows to various parts of the body. Lead most commonly enter the body through ingestion, and then travels to the lungs then swiftly through the blood stream hangeto other parts of the body. Lead does not change form once it enter the body. Lead in the blood stream tzzravels to the soft tissues of the body, such as the liver, kidneys, lungs, brain, spleen, muscles, and heart.(Adeniji., 2004).

Lead is a highly poisonous metal (regardless if inhaled or swallowed), affecting almost every organ and system in the body. The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system. Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO_2) can cause nephropathy, and colic-like

abdominal pains. It may also cause weakness in fingers, wrists, or ankles. Lead exposure also causes small increases in blood pressure, particularly in middle-aged and older people and can cause anemia. Exposure to high lead levels can severely damage the brain and kidneys in adults or children and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. Chronic, high-level exposure have shown to reduce fertility in males.Lead also damages nervous connections (especially in young children) and cause blood and brain disorders. Lead poisoning typically results from ingestion of food or water contaminated with lead; but may also occur after accidental ingestion of contaminated soil.(Golub., 2005).

TREATMENT METHODS:

Methods of treatment in which the application of physical forces predominate areknownas unit operation. Methods of treatment in which the removal of contaminants is brought about by chemical or biological reactions arc known as unit processes .At the present time, unit operations and processes are grouped together to provide various levels of treatment known as preliminary primary, advanced primary, secondary (without or with removal) and advanced (ortertiary) treatment. In preliminary treatment, gross solids such as large objects, rags,and grit are removed that may damage equipment. In primary treatment, a physical operation, usually sedimentation, isused to remove the floating and settle able materials found in wastewater. For advanced primary treatment, chemical are added to enhance the removal of suspended solids and to a lesser extent dissolved solids.In secondary treatment, biological and chemical processes are used to remove of theorganic matter. 1n advanced treatment, additional combinations of unit operations and processes are used remove residual suspended solids and other constituents that are not reduced significantly by conventional secondary treatment (Faryal., 2003).

The present study document the Isolation and characterization of Heavy Metal Tolerant Bacteria From Effulent Water Collected in Thandalam Lake Ranipet

Materials and methods

Collection of sample:

The effluent waste water collected from Thandalam lake, Ranipet. The water sample were collected in sterile glass bottle.

Screening and isolation of bacteria:

1 ml of sample was transferred into 99ml of distilled water and furthurdilution were made with 9 ml of water up to 7 dillution. The water was serially diluted and plated on Nutrient agar

plates. The plates were incubated at 37°C for 24 hours. After the number of Colony Forming Unit (CFU) were counted.(Selvi *et al*., 2012).

Identification and characterization of bacteria:

All the selected colonies were screened on MacConkey agar, EMB agar, and cetrimide agar. The shape and colors of the colonies were examined under the microscope after Gram staining, Isolates were biochemically analyzed for the activities of Oxidase, Catalase, MR- VP test, Motility, Indole test.(Selvi *et al.,* 2012).

Colony characterization:

The effluent water was inoculated in nutrient agar plate by using streak plate method. Plates were incubated in 37°C for 24 hours.

Gram staining:

Using sterile technique a smear was prepared from each of the isolated organisms. Then the smear was heat fixed. It was flooded with crystal violet and allowed to stand for one minute, then washed with tap water to remove the excess stain. The smear was again flooded with the Gram's Iodine and allowed to stand for one minute, then washed with tap water. It was then decolorized with 95% ethyl alcohol and washed immediately with tap water. Then finally counter stain with saffranin was added to the smear and allowed to stand for one minute and washed with tap water. The slide was air dried and observe under oil immersion objective.(Potter and Beth *et al.,* 2008)

Selective media:

The tested colonies are streaked in different selective media like EMB agar, Macconkey agar, Cetrimide agar. Plares were incubated in 37°C for 24 hours. After incubation colonies were observed.

Spore staining:

Using aseptic technique, a smear was prepared and the smear was fixed with gentle heat. The smear was covered with malachite green and exposed with continuous steaming to 5 minutes. While steaming evaporation of stain showed avoided. Then slide was washed with running tap water. Then the smear was covered with saffranin for one minute and washed with running tap water.

Catalase test:

Two ml of hydrogen peroxide (H_2O_2) was taken in a 8 ml tube. The isolates was introduced with the help of the tooth pick touched the sides of the test tube. The release of air bubbles observed within 10 seconds. (Potteand Beth *et al.,* 2008)

Oxidase test:

Oxidase disc was placed on clean glass slide. With the help of glass rod, the test organisms was picked by touching the colony from the plate and immediately pressed on the surtrface of the disc within 20 seconds the results were noted.(Potter and Beth *et al.,* 2008)

Indole test

The Indole test was performed to study the ability of the bacteria to break down tryptophan with tryptophanase. The liquid media contained 1% tryptone from the pancreatic digest of the milk protein casein. 500 µl of the Kovac's reagent were added to the tubes and shaken very gently. A red ring at the very top of the broth indicated a positive result for the presence of indole and tryptophanase activity.(Potter and Beth *et al.,* 2008).

MR-VP Test (Methyl red test and Voges-Proskauertest)

To prepare the methyl red and Voges-Proskauer tests, the bacteria were inoculated into a media of mixed acids and butanediol. The tubes were incubated for 48 hrs at 37^0C. 3 µlof the culture was transferred to a sterile tube to add two Barrit's Reagents for the Voges-Prokauer test, which looks for the fermentation of butanediol. 600 µL of alpha-napthol was added followed be the addition of 200µL of potassium hydroxide. A positive result was represented by a color change to red, indicating the presence of acetyl methyl carbinol. In the original test tube, 200µL of methyl red was added. The positive result was indicated by a color change to red, Which shows lactate, succinate, and or acetate were produced from the mixed acid fermentation.(Marcon *et al.,* 2009).

Phenol Red test:

A phenol red test looks at the bacteria's ability to ferment dextrose, lactose,mannitol or sucrose with the help of a P^H indicator. A Durham tube was also placed in the tube to collect any CO_2 that might have been produced. A yellow solution indicated that the bacteria are able to ferment sugars.(Camello *et al.,* 2009).

Coliform test:

Most Probable Number:

Presumptive test:

Distributes 5 ml of 1 x lactose broth (SSLB) each to 6 test tubes and 10 ml of 2x lactose broth (DSLB) to 3 test tubes. Introduces the Durham's tube and sterilze them at a time SSLB tubes as 0.1 ml. Label 3 DSLB test tubes as 10 ml, next 3 SSLB tubes as 5 ml the next. Inoculate 10 ml of water sample to each DSLB. Inoculate 10 ml of water sample to each DSLB. Inoculate 1 ml, o.1 ml, and 0.01 ml water sample to each of 3 SSLB, Lable correspondingly. Incubate the tubes at 37℃ and examine the gas formation in Durgam's tubes at 24hrs and 48 hrs. If gas is formed in 24 hrs (positive) or not until 48 hrs(doubtful test) proceed to confirmed test. (Yehuda kott 1996).

Conformed test:

Take a loopful of culture from the lactose broth tube from the highest dilution that still showed positive test and streak it on EMB agar plate and inoculate Brilliant green lactose broth. Incubate the plate for 24 hrs. Observe the gas formation in tubes. Coliforms are conformed by the formation of gas in lactose broth tube.All BGLB bubble gas were streaked to Eosine methylene blue agar(EMB). Incubated in 24 hrs at 35°C. After 24hrs the plates were show the Golden green metallic sheen colonies.(Yehuda kott 1996).

Completed test:

The colonies from the agar are inoculated back into lactose broth to demonstrate the production of acid and gas. (Yehuda kott 1996).

Detection of Heavy metals from water:

Analysis of heavy metals from effluent water collected in Thandalam Lake using Tamil Nadu Pollution Control Board (TNPCB), Advanced Environmental Laboratory, Chennai.

Analysis of physical and chemical examination of parameter of effluent water:

Analysis of parameters from effluent water using Tamil Nadu Water Supply and Drainage Board, District Level Water Testing Laboratory, Kancheepuram.

Collection of sample:

The effluent waste water collected from Thandalamlake, Ranipet. The water sample were collected in sterile glass bottle shown in the Figure: 1.

Screening and isolation of bacteria:

1 ml of sample was transferred into 99ml of distilled water and further dilution were made with 9 ml of water up to 7 dillution. The water was serially diluted and plated on Nutrient agar plates. The plates were incubated at 37°C for 24 hours. After the number of Colony Forming Unit (CFU) were counted. The results show in the figure: 2

Identification and characterization of bacteria:

All the selected colonies were screened on MacConkey agar, EMB agar, and cetrimide agar. The shape and colors of the colonies were examined under the microscope after Gram staining, Isolates were biochemically analyzed for the activities of Oxidase, Catalase, MR- VP test, Motility, Indole test.

Gram staining:

Using sterile technique a smear was prepared from each of the isolated organisms. Then the smear was heat fixed. It was flooded with crystal violet and allowed to stand for one minute, then washed with tap water to remove the excess stain. The smear was again flooded with the Gram's Iodine and allowed to stand for one minute, then washed with tap water. It was then decolorized with 95% ethyl alcohol and washed immediately with tap water. Then finally counter stain with saffranin was added to the smear and allowed to stand for one minute and washed with tap water. The slide was air dried and observe under oil immersion objective. The result show in Figure: 3 and Table: 1.

Selective media:

The tested colonies are streaked in different selective Media like EMB agar, Macconkey agar, Cetrimide agar. Plares were incubated in 37°C for 24 hours. After incubation green metallic sheen colonies are formed in EMB agar and no growth in both Macckonkey and cetrimide agar. The result show in Figure: 4

Spore staining: Gram positive strains were used for spore staining. Gram positive strains produced Endo spore formation. Result show in Figure: 5 and Table 1.

Catalase test:

Two ml of hydrogen peroxide (H_2O_2) was taken in a 8 ml tube. The isolates was introduced with the hrlp of the tooth pick touched the sides of the test tube. The release of air bubbles observed within 10 seconds.Result show in Table 1.

Oxidase test: Oxidase disc was placed on clean glass slide. With the help of glass rod, the test organisms was picked by touching the colony from the plate and immediately pressed on the surtrface of the disc within 20 seconds the results were noted. Result show in Table 1.

Indole test

The Indole test was performed to study the ability of the bacteria to break down tryptophan with tryptophanase. The liquid media contained 1% tryptone from the pancreatic digest of the milk protein casein. 500 µlof the Kovac's reagent were added to the tubes and shaken very gently. A red ring at the very top of the broth indicated a positive result for the presence of indole and tryptophanase activity. Result show in Table 1.

MR-VP Test (Methyl red test and Voges-Proskauertest)

To prepare the methyl red and Voges-Proskauer tests, the bacteria were inoculated into a media of mixed acids and butanediol. The tubes were incubated for 48 hrs at 37^0C. 3 µl of the culture was transferred to a sterile tube to add two Barrit's Reagents for the Voges-Prokauer test, which looks for the fermentation of butanediol. 600 µ of alpha-napthol was added followed be the addition of 200µl of potassium hydroxide. A positive result was represented by a color change to red, indicating the presence of acetyl methyl carbinol. In the original test tube, 200µl of methyl red was added. The positive result was indicated by a color change to red, Which shows lactate, succinate, and or acetate were produced from the mixed acid fermentation.Result show in Table 1.

Phenol Red test:

A phenol red test looks at the bacteria's ability to ferment dextrose, lactose,mannitol or sucrose with the help of a pH indicator. A Durham tube was also placed in the tube to collect any CO_2 that might have been produced. A yellow solution indicated that the bacteria are able to ferment sugars.The Result show in Table 1 and Figure 6.

Coliform test:

Most Probable Number

Presumptive test:

Distributes 5 ml of 1 x lactose broth (SSLB) each to 6 test tubes and 10 ml of 2x lactose broth (DSLB) to 3 test tubes. Introduces the Durham's tube and sterilze them at a time SSLB tubes as 0.1 ml. Label 3 DSLB test tubes as 10 ml, next 3 SSLB tubes as 5 ml the next. Inoculate 10 ml of water sample to each DSLB. Inoculate 10 ml of water sample to each DSLB. Inoculate 1 ml, o.1 ml, and 0.01 ml water sample to each of 3 SSLB, Lable correspondingly. Incubate the tubes at

37°C and examine the gas formation in Durgam's tubes at 24hrs and 48 hrs. After 24 hrs the gas was formed in the test tubes. The Result show in Table 2 and Figure 7.

Conformed test:

Take a loopful of culture from the lactose broth tube from the highest dilution that still showed positive test and streak it on EMB agar plate and inoculate Brilliant green lactose broth. Incubate the plate for 24 hrs. Observe the gas formation in tubes. Coliforms are conformed by the formation of gas in lactose broth tube. All BGLB bubble gas were streaked to Eosine methylene blue agar(EMB). Incubated in 24 hrs at 35°C. After 24hrs the plates were show the (Golden green metallic sheen colonies).The Result show in Table 3 and Figure 8.

Completed test:

The colonies from the agar are inoculated back into lactose broth to demonstrate the production of acid and gas. The gas was formed in test tubes. The Result show in Figure 9.

Detection of Heavy metal from water:

Analysis of heavy metal from effluent water using Tamil Nadu Pollution Control Board

(TNPCB), Advanced Environmental Laboratory, Chennai. The result show in Table 4.

Analysis of physical and chemical examination of parameter of effluent water:

Analysis of parameters from effluent water using Tamil Nadu Water Supply and Drainage Board, District Level Water Testing Laboratory, Kancheepuram.The result show in Table 5.

TABLE 1: BIOCHEMICAL TEST

Morphological / Physiological / Biochemical characteristics	Isolation 1	Isolation 2	Isolation 3	Isolation 4	Isolation 5	Isolation 6	Isolation 7	Isolation 8	Isolation 9	Isolation 10
Gram reaction	-	-	+	-	+	-	-	+	-	-
Motility	+	+	-	+	-	+	+	-	+	+
Spore staining	-	-	+	+	+	+	-	+	+	-
Oxidase test	+	+	-	-	-	-	+	-	-	+
Catalase test	+	+	+	-	+	-	+	+	-	+
Indole test	-	-		+		+	-		+	-
Methyl red Test	-	-	+	+	+	+	-	+	+	-
Voges-Proskauer Test	-	-		-		-	-		-	-
Glucose	A	A	A	A+G	A	A+G	A	A	A+G	A
lactose	-	-	-	+	-	+	-	-	+	-
Mannose	+	+	-	+	-	+	+	-	+	+
Sucrose	-	-	+	+	+	+	-	+	+	-

TABLE 2: PRESUMPTIVE TEST

NO. OF TUBES	SSLB (0.1ml)	SSLB (1ml)	DSLB (10 ml)
1	+	–	+
2	–	–	+
3	–	+	+
4	–	+	+
5	–	+	+

RESULT: 1: 3: 5 (19/ 100ml)

SSLB- Single Strength Lactose Broth

DSLB-Double Strength Lactose Broth

Table 3: CONFORMED TEST

NO. OF POSITIVE TUBES	SSLB (0.1ml)	SSLB (1ml)	DSLB (10 ml)
1	+	–	+
2	–	–	–
3	–	+	+
4	–	–	–
5	–	+	+

RESULT: 1: 2: 3 (12/ 100 ml)

Table 4: DETECTION OF HEAVY METALS FROM EFFULENT WATER

TAMIL NADU POLLUTION CONTROL BOARD

Advanced Environmental Laborastory, Chennai-32.

S.NO	Parameters	Unit	Value
1	Total chromium	mg/L	<0.01
2	Copper	mg/L	<0.0015
3	Zinc	mg/L	0.16
4	Lead	m/L	1.23
5	Nickel	mg/L	0.58
6	Cadmium	mg/L	<0.0008

<- Indicates less than minimum detection limit

Table 5: ANALYSIS OF PHYSICAL AND CHEMICAL EXAMINATION OF

EFFLUENT WATER

TAMILNADU WATER SUPPLY AND DRAINGE BOARD

District Level Water Testing Laboratory, Kancheepuram

Parameters	Sample No. 20637
PHYSICAL EXAMINATION	
Appearance	Pinkish Colour
Odour	Oil and Leather smell
Turbidity NTU	180.0
Total Dissolved solids	**4150**
Electrical conductivity micS/cm	**5930**
CHEMICAL EXAMINATION	
pH	7.96
Alkalinity pH as CaCo3	0
Alkalinity Total CaCo3	624
Total Hardness as CaCo3	500
Calcium as Ca	112
Magnesium as Mg	53
Sodium as Na	-

Potassium as K	-
Iron Total as Fe	0.00
Manganese as Mn	-
Free Ammonia as NH3	3.00
Nitrite as NO2	0.50
Nitrate as NO3	42
Chloride as Cl	1150
Fluoride as F	1.0
Phosphate as PO4	0.20
Tidy's test	0.9

FIGURES:

Figure 1: THANDALAM LAKE

Figure 2: SCREENING OF BACTERIA

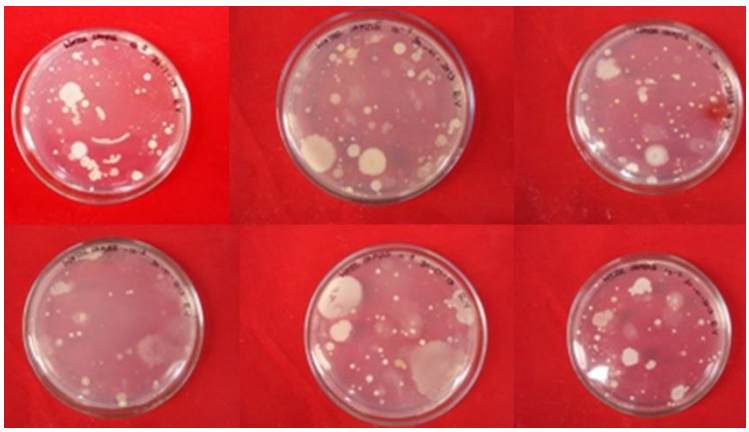


Figure 3: GRAM POSITIVE

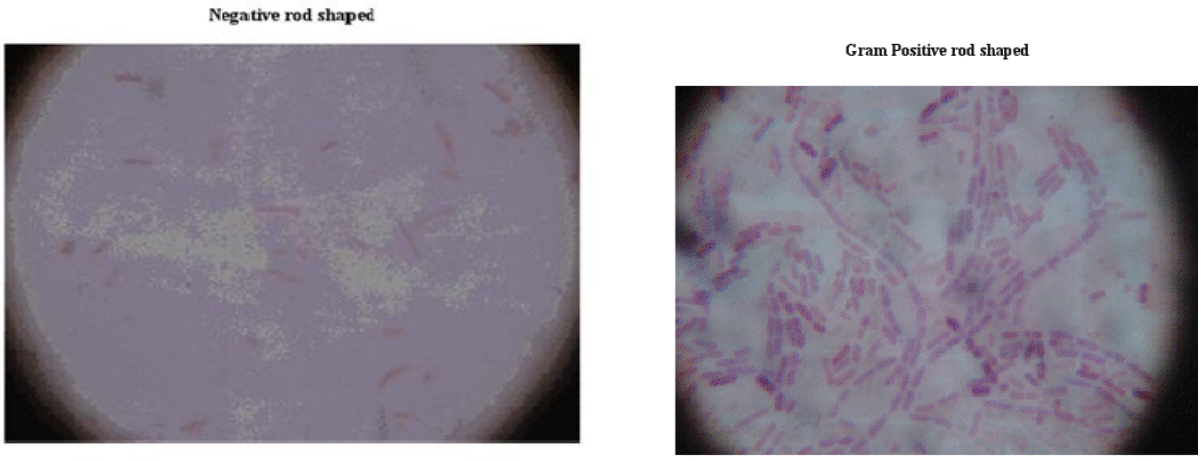


Figure 4: SELECTIVE MEDIA

EMB AGAR

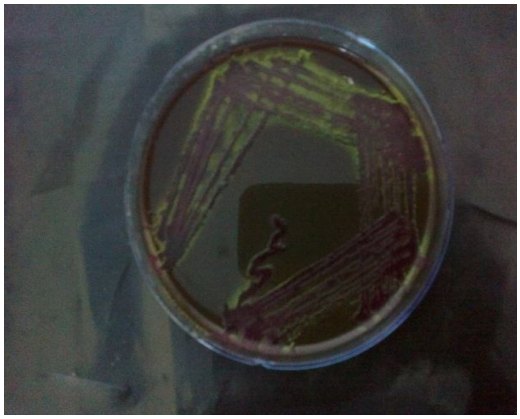


Figure 5: SPORE STAINING

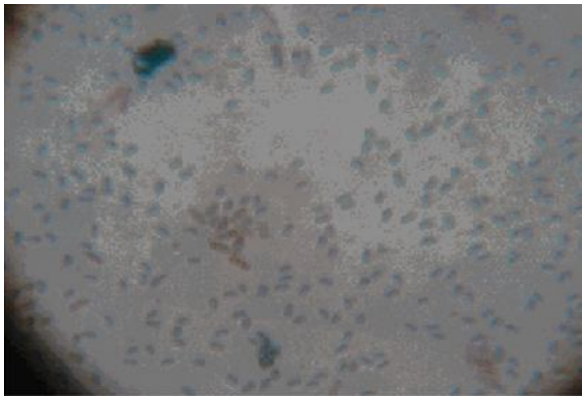


Figure 6: ACID PRODUCTION TEST

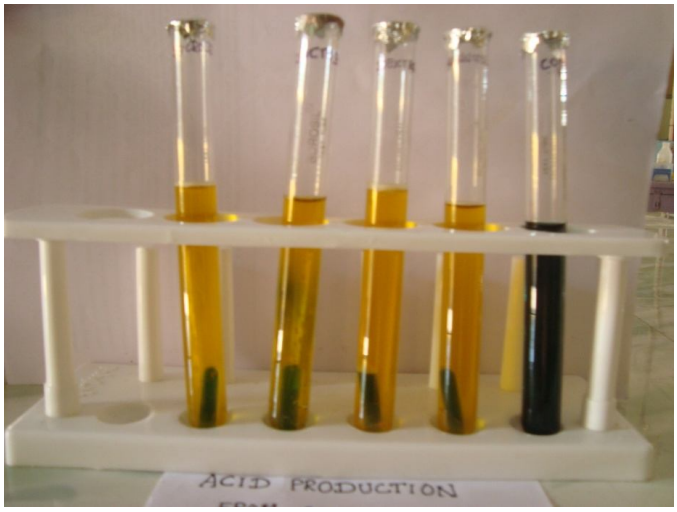


Figure 7: COLIFORM TEST

Presumptive test

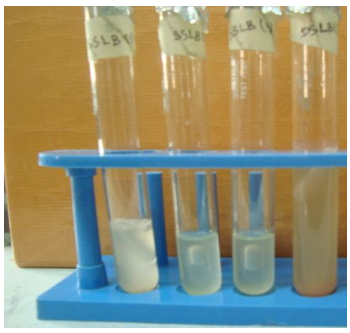
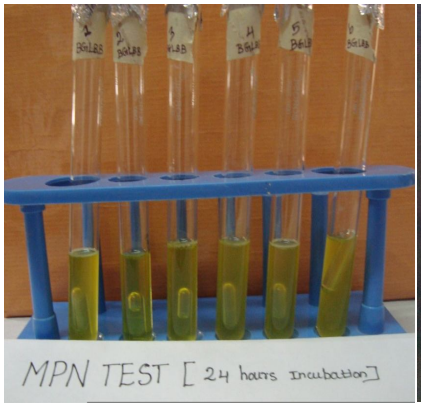


Figure 8: CONFORMED TEST

EMB AGAR



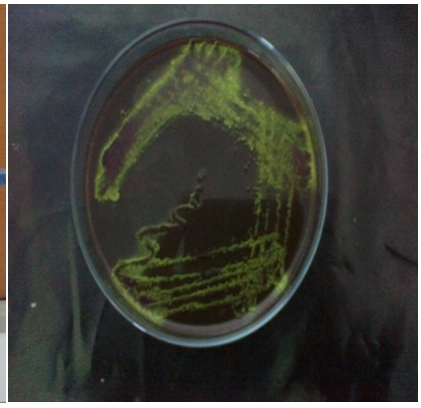
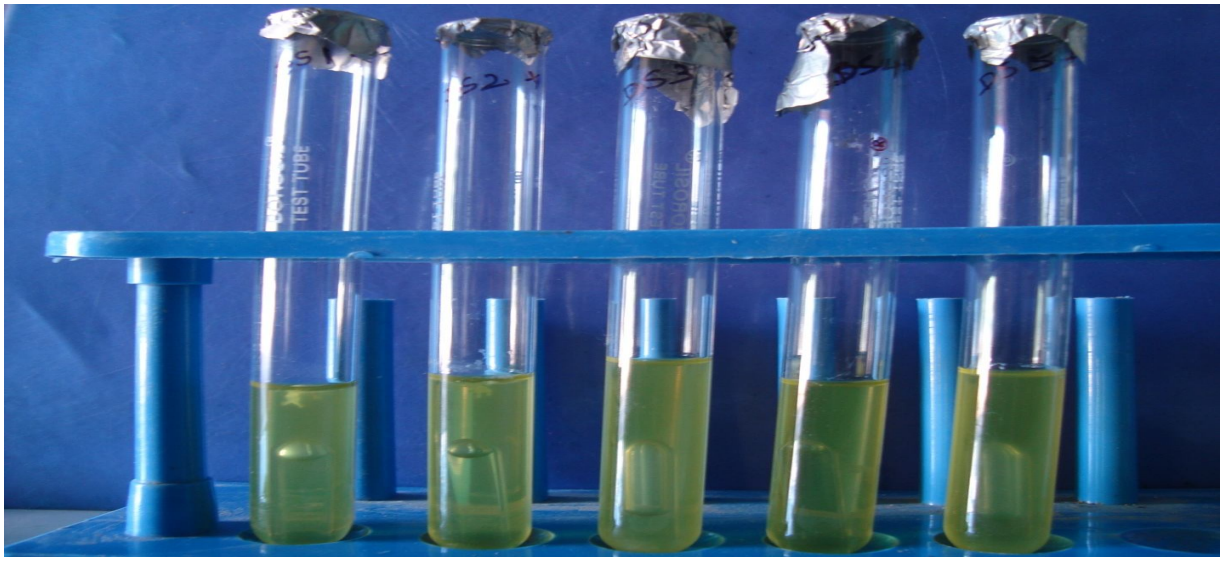


Figure 9: COMPLETED TEST



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