COMPRATIVE STUDY OF MICROORGANISM COLLECTED FROM WATER SAMPLE OF TWO DIFFERENT PONDS, UPARWARA, RAIPUR CHHATTISGARH INDIA

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Abstract

There are many thousands of types of bacteria and microorganisms found in aquatic ecosystems, including your personal pond. The main aquatic groups include arthropods, bacteria, protozoa, hydras, fungi, phytoplankton, and algae. A pond is a small area of still, fresh water. Bacteria rarely live alone but in communities with other bacteria. This is true both in the environment and in and on our bodies. Identification and characterization of microorganisms is a key part of the management of food safety and quality, tracing contaminants and troubleshooting problems such as spoilage. Knowing how to identify bacteria or other unknown microorganisms that you have encountered can help to assess whether it poses a safety/spoilage concern or is likely to be heat resistant. Most of the currently known species of bacteria have been identified using traditional microbiological techniques such as the gram stain reaction, morphology, and metabolic reactions. The aim of this study was to isolate and identify pond bacteria. Colonies need to be well isolated from two different ponds of uparawara village of Chhattisgarh in India. Observing colony morphology is an important skill we used in the microbiology laboratory to identify

microorganisms using traditional microbiological techniques such as the gram stain reaction to observe the characteristic shape, size, color, surface appearance, and texture.

Keywords: Microorganism; water

Introduction

Water is an essential nutrient which plays an important role in digestion, absorption of food and elimination of waste products by urine. The aquatic systems are mostly dominated by bacteria and fungi and in the natural environments micro-organisms have very specific roles with regard to the recycling of materials and purification of water. Presence of coliform bacteria in the water indicates the fecal pollution of water. *almonella, Acinetobacter, Chromobacterium, Alcaligens, Flavobacterium, Staphylococcu saureus, Pseudomonas aeruginosa, Clostridium botulinum, Vibrio cholerae* and *Escherichia coli* are the main human pathogens responsible for water contamination. This confirms the presence of *E. coli* in the water bodies giving a definite proof of fecal pollution thus not being suitable for bathing and drinking.(Upasana Bhumbla, et. al. 2020)

According to World Health Organization (WHO), most children from 3.4 million infected people die from water-related diseases each year Based on reports of United Nations Children's Fund (UNICEF 2014) assessment, 4000 children die each day because of contaminated water. Meanwhile, WHO (2010) reports that over 2.6 billion people lack access to clean water. Among them, about 2.2 million deaths annually, of which 1.4 million are in children (Nuraiffa Syazwi et. al 2018).

Many sources of water in earth. In this A pond is a small area of still, fresh water. It is different from a river or a stream because it does not have moving water and it differs from a lake because it has a small area and is no more than around 1.8m deep. In ponds, the types of bacteria and microorganisms found differs depending on the type of water body – perhaps predictably, natural ponds contain a greater diversity of these beneficial organisms than do most man-made ponds.(Shiva Ghaderifar et. al 2018).

There are many thousands of types of bacteria and microorganisms found in aquatic ecosystems, including your personal pond. The main aquatic groups include arthropods, bacteria, protozoa, hydras, fungi, phytoplankton, and algae. Organisms found in pond water are quite diverse. The abundance of nutrients in ponds makes them ideal environments to host multiple organisms. These organisms can either be macroscopic (which can be seen with the naked eye) or microscopic (which can only be seen with a help of a microscope).(Ankita Khan et.al 2019) Microorganisms have major roles in pond culture, particularly with respect to productivity, nutrient cycling, the nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent. Analysis of the complex food webs in ponds, combined with measurements of primary productivity, C cycling through bacteria, Zooplankton and meiofaunal biomass changes and nitrogen cycling will provide the basic data for generating a model to describe and predict fish productivity in ponds.(Moumita Sarkar et.al. 2019)

Organisms present in pond water belong to different kingdoms and groups. Protozoa, Spirogyra, (blue-green algae) and Hydra are the microorganisms found in pond water commonly. For instance, there are various organisms in Kingdom Animalia that occupy

aquatic habitats. In addition, organisms in Kingdom Plantae also occupy aquatic habitats. Moreover, protists can also be found in pond water. Initially, these organisms belonged to Kingdom Protista; however, this kingdom is currently regarded as obsolete. Within every drop of pond water lurks an invisible world, alive with an amazing variety of microscopic creatures. You can find simple life forms such as bacteria, great oxygen-producers like algae, all kinds of alien-like protozoans, and cute microscopic animals like water bears.(Ruangpan, L., & Tendencia, E. A. (2004).Bacterial isolation, purification and identification are the first steps to bacteriological studies. Isolation is done to obtain pure bacterial cultures.(Ruangpan, L., & Tendencia, E. A. (2004).

Isolation of bacterial contaminants is performed by standard microbiological techniques and battery of biochemical reactions. Various biochemical reactions such as IMViC, Urease, Nitrate, Catalase, H_2S production, Sugar fermentation tests were identified for the identification of bacterial isolates. (Safia Arbab et. al 2021)To obtain a pure bacterial culture is the first step to bacterial identification. Pure culture is essential in the study of the morphology, physiology, biochemical characteristics, and susceptibility to antimicrobial agents of a particular bacterial strain. (Aakanchha Jain, Richa Jain & Sourabh Jain ,2020). Generally, they involve a combination of Gram staining, culture, and biochemical tests. As the different tests are carried out, the results obtained narrow the possible options until an identification is obtained. Well known examples of phenotypic tests include API strips or VITEK.(Miskiah fadzilah Ghazali et. al 2018)

After the identification of the microorganisms, depending on its shape and nature many tests related to it can be done on it. The characteristics of the microorganisms determine the the tests to be performed on it. Like An antibiotic sensitivity test is used to help find the best treatment for a bacterial infection. It may also be used to find out which treatment will work best on certain fungal infections. (Marlon L. Bayot; & Bradley N. Bragg. 2022) An enzyme marker is a blood test to measure enzymes, proteins in your blood that can indicate tissue damage or disease. The catalase test facilitates the detection of this enzyme in bacteria,etc.(Tadayuki Iwase et.al 2013)

Materials and Methodology

Sampling site and sample collection

For these experiment ,we selected a village named Uparwara in new Raipur area. where the number of pondswas slightly more than others nearby places. First of all we went to Uparwara village and inspected the environment there. then two ponds were selected from the ponds presents there, which are used by the people living there for daily life. Then brought water samples from those two ponds.

Serial dilution

Serial dilution is a process through which the concentration of an organism, bacteria in this example, is systematically reduced through successive resuspension in fixed volumes of liquid diluent. Usually the volume of the diluent is a multiple of 10 to facilitate logarithmic reduction of the sample organism. For serial dilutions we used 10 test tubes. In 5 test tubes we took water from Ramsagar pond and in 5 from Sheetla pond. The number of test tubes for serial dilution depends on the number of plates we want to grow bacteria in.

Media Preparation

Media preparation products provide an optimum growing environment for culturing microbiological organisms. Media preparation products differ based on the types of nutrients and their combination. In preparing a culture medium for any microorganism, the primary goal is to provide a balanced mixture of the required nutrients, at concentrations that will permit good growth. First of all, we made the NAM media then by pouring serial diluted water from the selected test tubes into the NAM plate, spread it with the help of a glass spreader with light hands. To make media plates, we needed 10 plates so that we could pour Ramsagar pond water in 5 plates and Sheetla pond water in 5. No. of plates can be increased or decreased according to bacterial growth. After solidifying we pack it well sealed with parafilm so that no external elements can enter it. Then we put those plates In the incubator growth for 2 to 3 days so that after growth we can subculture them.

Subculture

Sub culturing describes the transfer of microbes from one growth medium container, such as broth or agar, to another, and allowing the microbes to grow. Subculture is therefore used to produce a new culture with a lower density of cells than the originating culture, fresh nutrients and no toxic metabolites allowing continued growth of the cells without risk of cell death. Subculture is important for both proliferating. Sub culturing is also useful in keeping strains alive by transferring them to fresh growth medium. In this steps Label the agar slants and agar plates with the organism name by using the wax marking pencil.

Sterilize the inoculating loop by holding it in the blue portion of the flame until it gets hot red. Allow the loop to cool for 30- 40 sec or cool it by dipping it in a fresh agar plate. Touch the tip of the loop to the surface of a selected discrete colony or the agar streak plate or the pour plate. Remove the plug of the agar slants, grasp the plug with the little finger of the hand and pass the neck of the tube rapidly over the Bunsen burner flame. Insert loop into the subculture tube and inoculate in by drawing it lightly over the hard surface in a straight or zig-zag line and recap the tube. The neck of the subculture tube is flamed and the cotton plug is reinserted. Following inoculation, the loop is again flamed to destroy the remaining organisms. Incubate the cultures (in slants/Petri plates) at 25°C for 48–72 hours.

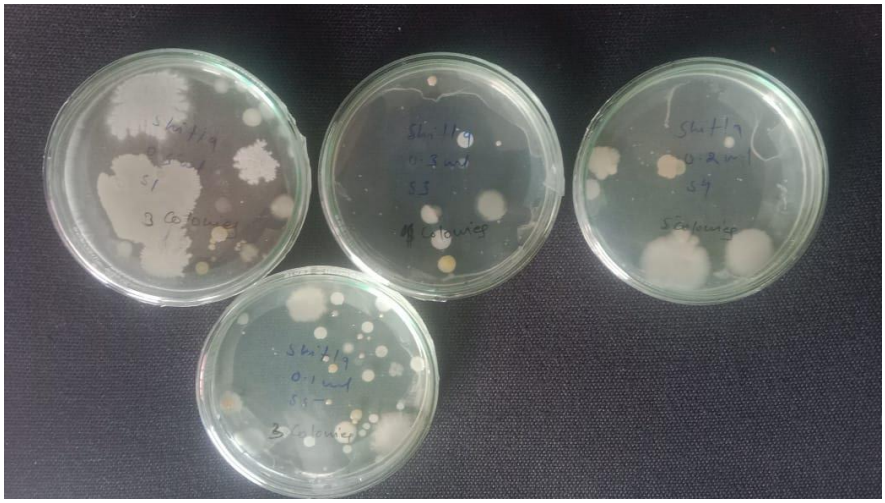
Identification of microorganisms through selective plating and Gram Staining

After placing in subculture incubation , the process of identification was further carried out by taking samples from plate in which the best growth came. For Gram staining, the smears of bacterial isolates were prepared and heat fixed. Then, they were gently flooded with crystal violet and was kept stand for 1 minute and gently washed with tap water. The same process was repeated with Gram's iodine. Further, they were decolorized with 95% ethyl alcohol and again gently washed with tap water. Then, samples were counter stained with safranine and washed with tap water. The samples were air dried and examined under oil immersion at 100x magnification.

Result and Discussion

This test has been done to know about the microbes of two ponds. To know about the numbers, shape, size and nature of microorganisms in the both ponds. while comparing after making the NAM plates, we found that the no.s of microbes in the plates of Sheetla

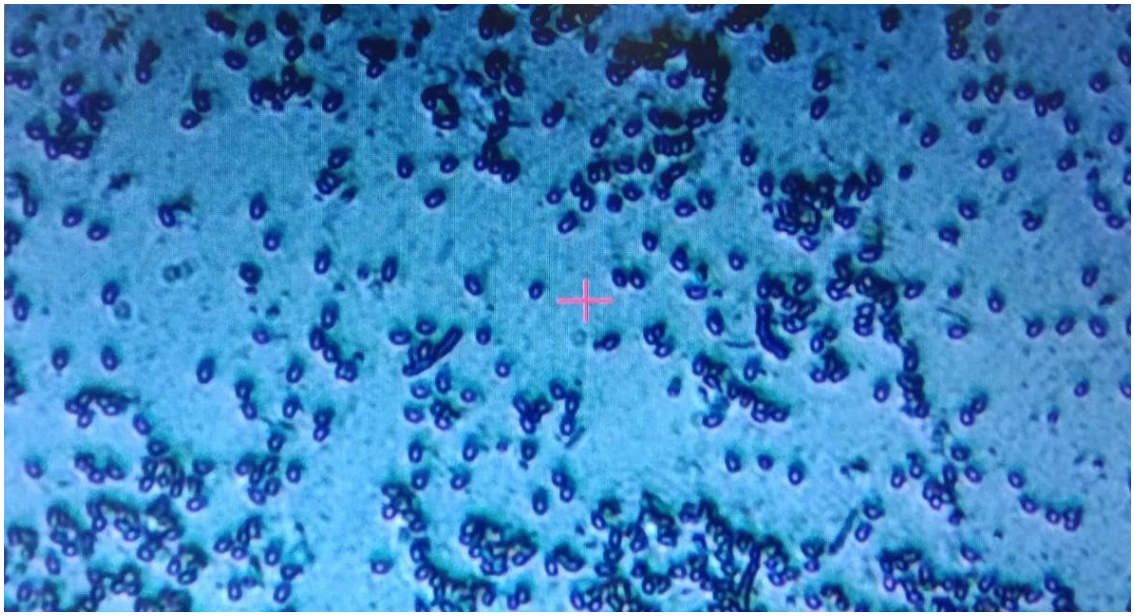
pond is less as compared to the plates of Ramsagar pond. We got 9 types of bacteria samples from Sheetla pond and 12 types from Ramsagar pond. We did complete culture of all the bacteria samples in different plates till their 1 to 9 & 1 to 12 no. gave for both ponds. By doing pure culture of microorganisms obtained from water sample of Sheetla pond, it was found that the growth of bacteria samples of plate no. 6 is more than that bacteria samples of other plates. Whose identify is in the form of "**Coccus"**. though this process pure culture of microorganisms of water sample of ramsagar pond was done. Increased growth was observed in plates no. 2,3 & 4 which is recognized as both "**Coccus ,Bacillus and mixed form of Coccobasillus".**



Fig; Pour plates no. 1,3,4,5

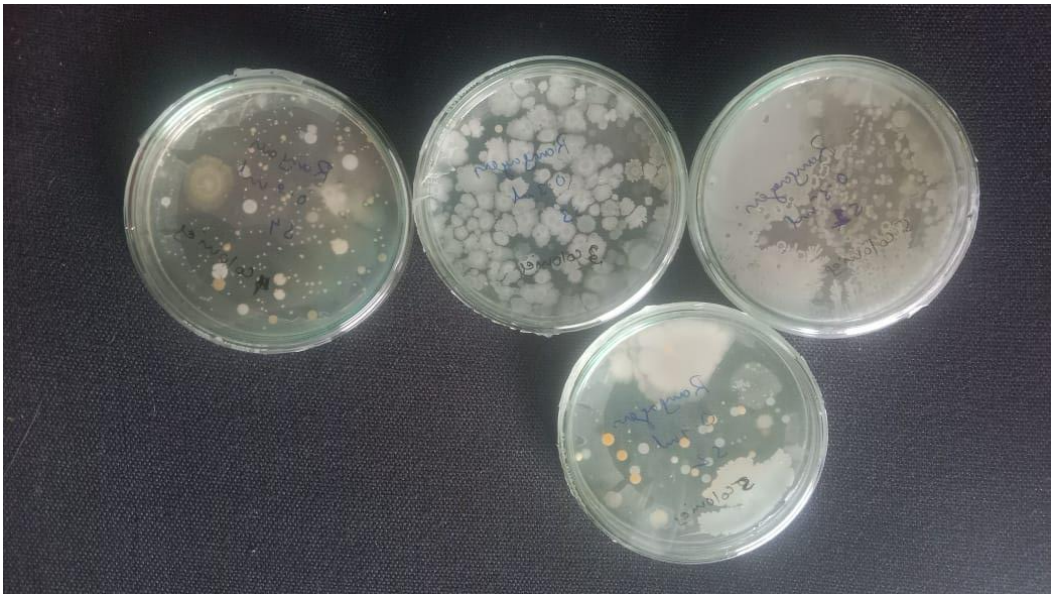
Fig: 01 (Growth of Microrganisms of Shitla pond water sample ,Uparwara Raipur Chhattisgarh,INDIA)

Fig; Streak plate no.6



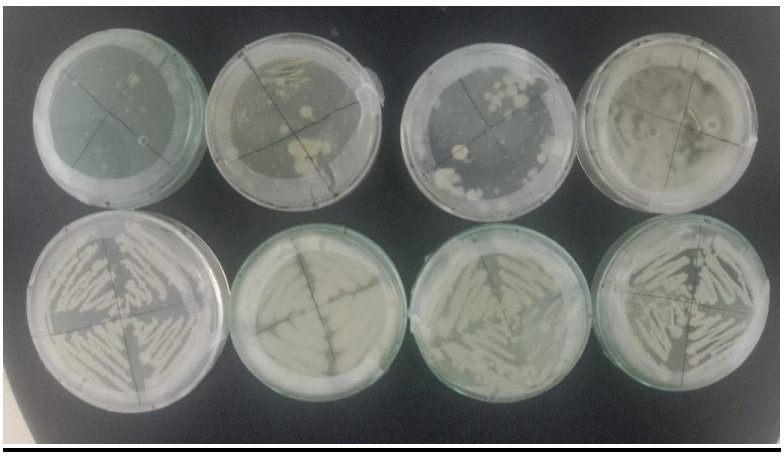
Fig; Gram positive ,Coccus "Round shaped (Monococcus, diplococcus, streptococcus, staphylococcus)

Fig: 02 (Pure culture of microorganisms and identified bacteria from Shitla pond water sample plate no. 06 Uparwara Raipur Chhattisgarh, INDIA)

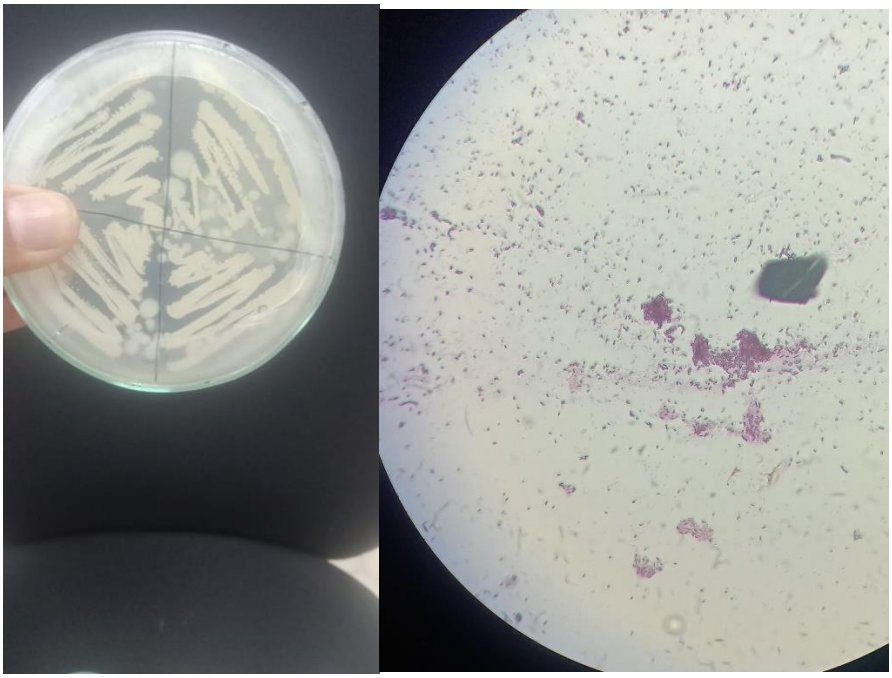


Fig; pour plates no. 1,2,3,4

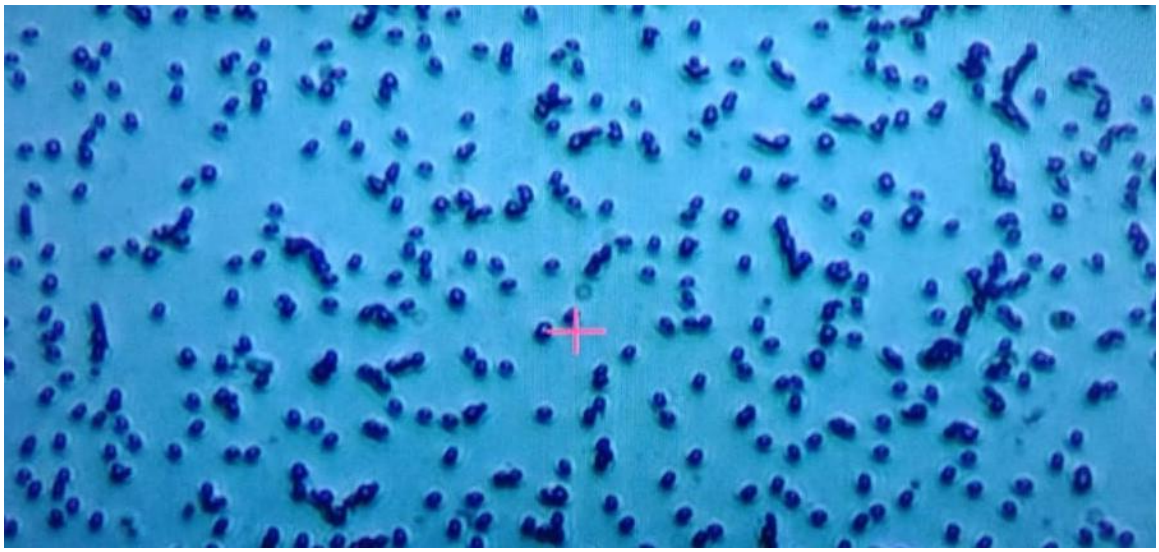
Fig: 03 (Growth of Microorganisms of Ramsagar pond water sample, Uparwara Raipur Chhattisgarh, INDIA)



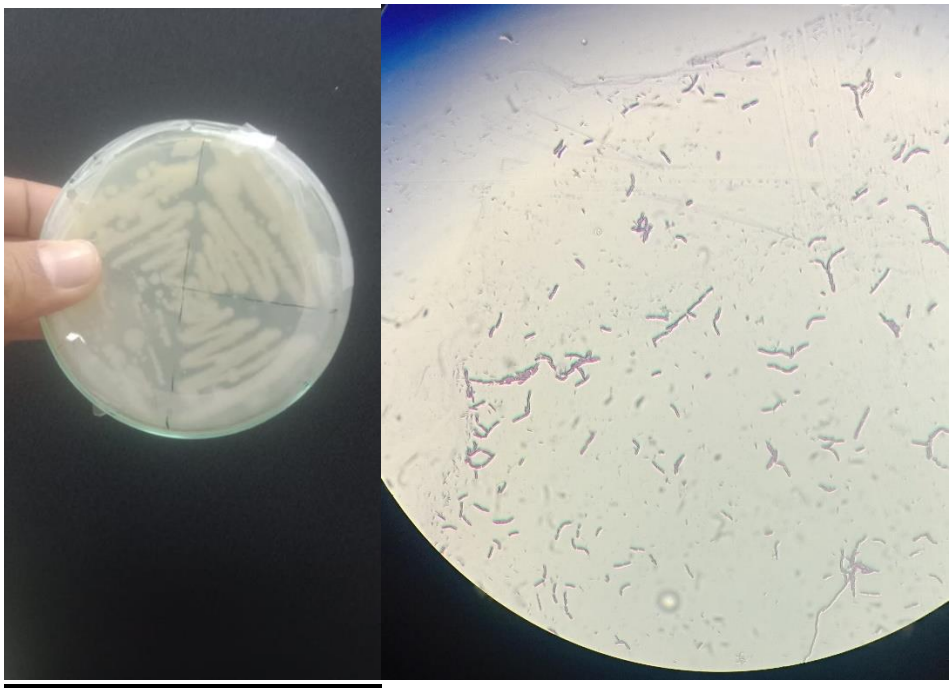
Fig; Streak plates no.1,2,3,4,5,6,7,8



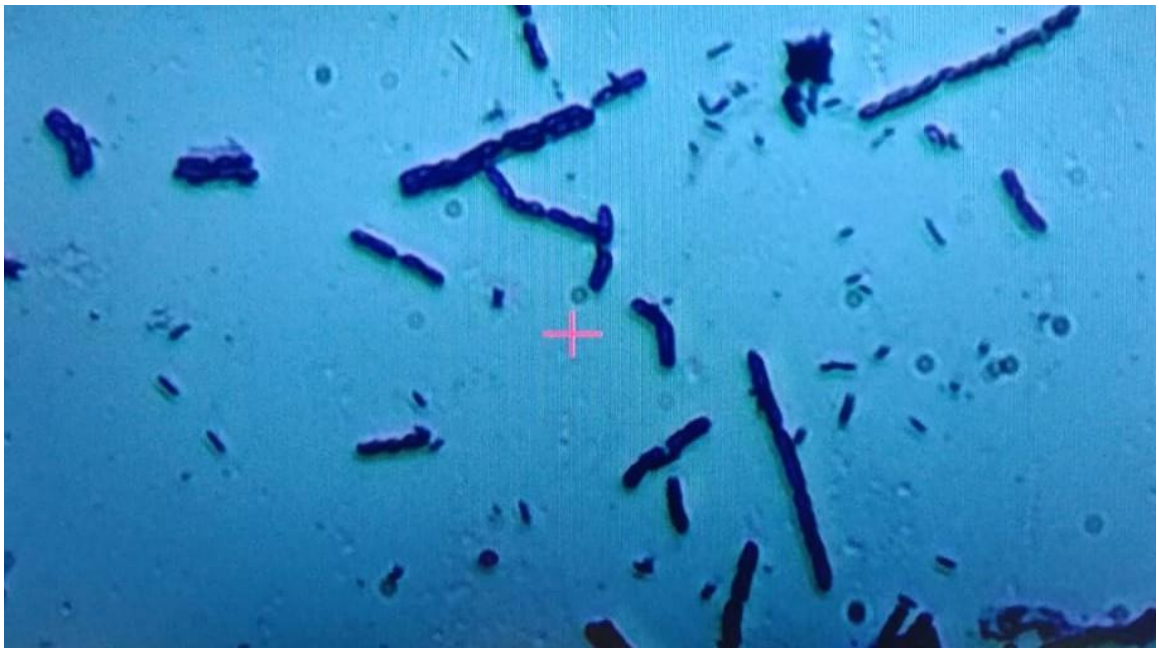
Fig; Streak pate no. 02 (Microscopic Pic)



Fig; Gram positive Coccus "Round shaped" (Monococcus, Diplococcus, streptococcus)

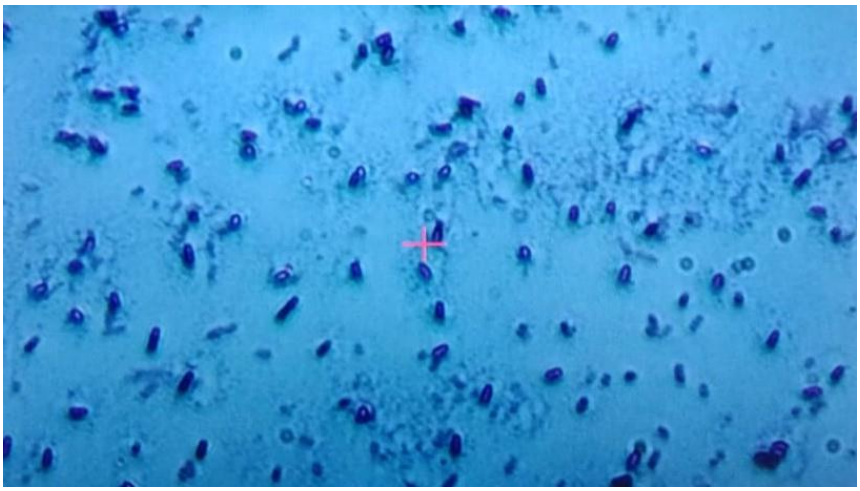


Fig; Strek plate no.03 (Microscopic)



Fig; Gram negative Bacillus "Rod Shaped" (Monobacillus, Diplobacillus, Coccobacillus, Streptobacillus)

Fig; Streak plate no.04



Fig; Gram positive Bacillus (Coccobasillus)

Fig: 04 (Pure culture of microorganisms and identified bacteria from ramsagar pond water sample plates no.2, 3 & 4 Uparwara Raipur Chhattisgarh, INDIA).

References

1. Abedin, Mohammad Zakerin, Md Sadiqur Rahman, Rubait Hasan, Jamiatul Husna Shathi, Laila Jarin, and Md Sifat Uz Zaman. "Isolation, identification, and antimicrobial profiling of bacteria from aquaculture fishes in pond water of Bangladesh." American. Journal of Pure and Applied BioSciences 2, no. 3 (2020): 39-50.
2. Abu, G. O., and A. C. Wondikom. "Isolation, characterization and antibiotic resistance profile studies of bacteria from an excavated pond in Port Harcourt

Metropolis, Nigeria." Journal of Applied Sciences and Environmental Management 22, no. 8 (2018): 1177-1184.

3. Adams, Craig, Y. Wang, K. Loftin, and M. Meyer. "Removal of antibiotics from surface and distilled water in conventional water treatment processes." Journal of environmental engineering 128, no. 3 (2002): 253-260.
4. Adeyemi, Folasade M., Olabisi O. Ojo, Anthony A. Badejo, Omotayo O. Oyedara, Janet O. Olaitan, Charles O. Adetunji, Daniel I. Hefft, Adeniyi A. Ogunjobi, and Sunday B. Akinde. "Integrated poultry-fish farming system encourages multidrug-resistant gram-negative bacteria dissemination in pond environment and fishes." Aquaculture 548 (2022): 737558.
5. Adhimi, Rim, Ghassan Tayh, Salma Ghariani, Sarra Chairat, Abdelmonem Chaouachi, Abdellatif Boudabous, and Karim Ben Slama. "Distribution, diversity and antibiotic resistance of Pseudomonas spp. isolated from the water dams in the north of Tunisia." Current Microbiology 79, no. 7 (2022): 188.
6. Al-Harbi, Ahmed H., and Naim Uddin. "Quantitative and qualitative studies on bacterial flora of hybrid tilapia (Oreochromis niloticus× O. aureus) cultured in earthen ponds in Saudi Arabia." Aquaculture Research 34, no. 1 (2003): 43-48.
7. Angenent, Largus T., Scott T. Kelley, Allison St Amand, Norman R. Pace, and Mark T. Hernandez. "Molecular identification of potential pathogens in water and air of a hospital therapy pool." Proceedings of the National Academy of Sciences 102, no. 13 (2005): 4860-4865.

8. Apun, Kasing, Asiah M. Yusof, and Kumbang Jugang. "Distribution of bacteria in tropical freshwater fish and ponds." International Journal of Environmental Health Research 9, no. 4 (1999): 285-292.
9. Barosa, Nourshamsia C., Beverly B. Amparado, Mariam C. Kabirun, and Anabella G. Villarino. "Antibiotic resistance profiling and evaluation of multiple antibiotic resistance (MAR) index of bacterial isolates from surface water of Lake Lanao, Philippines." International Journal of Pharma Medicine and Biological Sciences 9 (2020): 75-80.
10. Baumann, Paul. "Isolation of Acinetobacter from soil and water." Journal of bacteriology 96, no. 1 (1968): 39-42.
11. Botes, Marelize, Michéle de Kwaadsteniet, and Thomas Eugene Cloete. "Application of quantitative PCR for the detection of microorganisms in water." Analytical and bioanalytical chemistry 405 (2013): 91-108.
12. Buszewski, Bogusław, Agnieszka Rogowska, Paweł Pomastowski, Michał Złoch, and Viorica Railean-Plugaru. "Identification of microorganisms by modern analytical techniques." Journal of AOAC International 100, no. 6 (2017): 1607-1623.
13. Chakravarthy, S. Kalyana, E. V. V. Ramaprasad, E. Shobha, Ch Sasikala, and Ch V. Ramana. "Rhodoplanes piscinae sp. nov. isolated from pond water." International journal of systematic and evolutionary microbiology 62, no. Pt_12 (2012): 2828-2834.
14. Cooley, Michael B., Michele Jay-Russell, Edward R. Atwill, Diana Carychao, Kimberly Nguyen, Beatriz Quiñones, Ronak Patel et al. "Development of a robust

method for isolation of Shiga toxin-positive Escherichia coli (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California." PLoS One 8, no. 6 (2013): e65716.

15. Coşkun, Kübra Açıkalın, Semra Özçelik, Lütfi Tutar, Nazif Elaldı, and Yusuf Tutar. "Isolation and identification of free-living amoebae from tap water in Sivas, Turkey." BioMed research international 2013 (2013).
16. Costanzo, Simon D., John Murby, and John Bates. "Ecosystem response to antibiotics entering the aquatic environment." Marine pollution bulletin 51, no. 1-4 (2005): 218-223.
17. De Souza, M. P., A. Amini, M. A. Dojka, I. J. Pickering, S. C. Dawson, N. R. Pace, and N. Terry. "Identification and characterization of bacteria in a selenium-contaminated hypersaline evaporation pond." Applied and Environmental Microbiology 67, no. 9 (2001): 3785-3794.
18. Dekker, Linda, Thomas H. Osborne, and Joanne M. Santini. "Isolation and identification of cobalt-and caesium-resistant bacteria from a nuclear fuel storage pond." FEMS microbiology letters 359, no. 1 (2014): 81-84.
19. Douglas, Salome I., and Faith N. Isor. "Bacteriological investigation of pond water quality from Ogoniland, Nigeria." IOSR Journal of Environmental Science, Toxicology and Food Technology 9, no. 2 (2015): 36-41.
20. Douglas, Salome I., and Faith N. Isor. "Bacteriological investigation of pond water quality from Ogoniland, Nigeria." IOSR Journal of Environmental Science, Toxicology and Food Technology 9, no. 2 (2015): 36-41.

21. Edelstein, PAUL H. "Comparative study of selective media for isolation of Legionella pneumophila from potable water." Journal of Clinical Microbiology 16, no. 4 (1982): 697-699.
22. Ekundayo, F. O., K. A. Oyeniran, and A. D. Adedokun. "Antimicrobial activities of some streptomyces isolated from garden soil samples and fish pond water in FUTA." Journal of Bio-Science 22 (2014): 21-29.
23. Finton, Misti D., Roger Meisal, Davide Porcellato, Lin T. Brandal, and Bjørn-Arne Lindstedt. "Whole genome sequencing and characterization of multidrug-resistant (MDR) bacterial strains isolated from a Norwegian university campus pond." Frontiers in Microbiology 11 (2020): 1273.
24. Gandhi, Vikram Pal, and Anil Kumar. "Isolation and characterization of microcystin degrading bacteria from Holy Ponds in India." International Journal of Applied Sciences and Biotechnology 4, no. 4 (2016): 436-447.
25. Gholami-Borujeni, Fathollah, Kazem Naddafi, and Fatemeh Nejatzade-Barandozi. "Application of catalytic ozonation in treatment of dye from aquatic solutions." Desalination and Water Treatment 51, no. 34-36 (2013): 6545-6551.
26. Gu, Ganyu, Zhiyao Luo, Juan M. Cevallos-Cevallos, Paige Adams, George Vellidis, Anita Wright, and Ariena HC van Bruggen. "Factors affecting the occurrence of Escherichia coli O157 contamination in irrigation ponds on produce farms in the Suwannee River Watershed." Canadian journal of microbiology 59, no. 3 (2013): 175-182.
27. Guo, Mei-Ting, Qing-Bin Yuan, and Jian Yang. "Microbial selectivity of UV treatment on antibiotic-resistant heterotrophic bacteria in secondary effluents of a

municipal wastewater treatment plant." Water Research 47, no. 16 (2013): 6388-6394.

28. Heenatigala, P. P. M., and M. U. L. Fernando. "Occurrence of bacteria species responsible for vibriosis in shrimp pond culture systems in Sri Lanka and assessment of the suitable control measures." Sri Lanka Journal of Aquatic Sciences 21, no. 1 (2016).
29. Heenatigala, P. P. M., and M. U. L. Fernando. "Occurrence of bacteria species responsible for vibriosis in shrimp pond culture systems in Sri Lanka and assessment of the suitable control measures." Sri Lanka Journal of Aquatic Sciences 21, no. 1 (2016).
30. Homklin, Supreeda, Say Kee Ong, and Tawan Limpiyakorn. "Degradation of 17α-methyltestosterone by Rhodococcus sp. and Nocardioides sp. isolated from a masculinizing pond of Nile tilapia fry." Journal of hazardous materials 221 (2012): 35-44.
31. Huysmans, K. Davis, and W. T. Frankenberger Jr. "Evolution of trimethylarsine by a Penicillium sp. isolated from agricultural evaporation pond water." Science of the total environment 105 (1991): 13-28.
32. Kapardar, Raj Kishor, Ravi Ranjan, Amit Grover, Munish Puri, and Rakesh Sharma. "Identification and characterization of genes conferring salt tolerance to Escherichia coli from pond water metagenome." Bioresource technology 101, no. 11 (2010): 3917-3924.

33. Keon, Meaghan R., Michael J. McKie, Liz Taylor-Edmonds, and Robert C. Andrews. "Evaluation of enzyme activity for monitoring biofiltration performance in drinking water treatment." Water Research 205 (2021): 117636.
34. Khanom, Umme Shahina, Sabrina Sharmeen, J. Ferdous, Wahhida Shumi, Arifin Abdu, Hazandi Abdul Hamid, and Md Aktar Hossain. "Determination of pond water quality for aquaculture and ecosystem management." Jouranl of Food, Agriculture and Environment 12, no. 3-4 (2014): 389-394.
35. Kovalakova, Pavla, Leslie Cizmas, Thomas J. McDonald, Blahoslav Marsalek, Mingbao Feng, and Virender K. Sharma. "Occurrence and toxicity of antibiotics in the aquatic environment: A review." Chemosphere 251 (2020): 126351.
36. Laurila, Jaana, Virpi Ahola, Ari Lehtinen, Tiina Joutsjoki, Asko Hannukkala, Anne Rahkonen, and Minna Pirhonen. "Characterization of Dickeya strains isolated from potato and river water samples in Finland." European Journal of Plant Pathology 122 (2008): 213-225.
37. Mahdavi, Hamed, Vinay Prasad, Yang Liu, and Ania C. Ulrich. "In situ biodegradation of naphthenic acids in oil sands tailings pond water using indigenous algae–bacteria consortium." Bioresource technology 187 (2015): 97-105.
38. Maleki, Nasim, Soheila Kashanian, Erfan Maleki, and Maryam Nazari. "A novel enzyme based biosensor for catechol detection in water samples using artificial neural network." Biochemical engineering journal 128 (2017): 1-11.
39. Manage, Pathmalal M., Christine Edwards, Brajesh K. Singh, and Linda A. Lawton. "Isolation and identification of novel microcystin-degrading

bacteria." Applied and Environmental Microbiology 75, no. 21 (2009): 6924-6928.

40. Moss, Shaun M., Brad R. LeaMaster, and James N. Sweeney. "Relative abundance and species composition of gram-negative, aerobic bacteria associated with the gut of juvenile white shrimp Litopenaeus vannamei reared in oligotrophic well water and eutrophic pond water." Journal of the world aquaculture society 31, no. 2 (2000): 255-263.
41. Nadella, Ranjit Kumar, Satyen Kumar Panda, B. Madhusudana Rao, K. Pani Prasad, R. P. Raman, and Mukteswar Prasad Mothadaka. "Antibiotic resistance of culturable heterotrophic bacteria isolated from shrimp (Penaeus vannamei) aquaculture ponds." Marine Pollution Bulletin 172 (2021): 112887.
42. Naviner, Magali, Laurence Gordon, Etienne Giraud, Martine Denis, Catherine Mangion, Hervé Le Bris, and Jean-Pierre Ganière. "Antimicrobial resistance of Aeromonas spp. isolated from the growth pond to the commercial product in a rainbow trout farm following a flumequine treatment." Aquaculture 315, no. 3-4 (2011): 236-241.
43. Niemi, M. A. A. R. I. T., M. E. R. V. I. Sibakov, and S. E. P. P. O. Niemela. "Antibiotic resistance among different species of fecal coliforms isolated from water samples." Applied and Environmental Microbiology 45, no. 1 (1983): 79-83.
44. Njoku, O. E., O. K. Agwa, and A. A. Ibiene. "An investigation of the microbiological and physicochemical profile of some fish pond water within the

Niger Delta region of Nigeria." African journal of food Science 9, no. 3 (2015): 155-162.

45. Pakingking, Rolando, Peter Palma, and Roselyn Usero. "Quantitative and qualitative analyses of the bacterial microbiota of tilapia (Oreochromis niloticus) cultured in earthen ponds in the Philippines." World Journal of Microbiology and Biotechnology 31 (2015): 265-275.