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Isolation and Examination of Pigment Producing Bacteria from Bhopal Lakes and Surrounding Ecosystem

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ABSTRACT

In the present study pigment and non–pigment producing bacteria were isolated from lakes and surrounding areas of Bhopal. The bacterial strains isolated on culture media, identified and purified for confirmation. The bacteria isolated were identified based on their phenotypic and biochemical characteristics. Most of the strains isolated possess yellow, orange and red pigmentation. The isolated strains developed in pure culture form and maximum pigment production determined at different pH, temperature and NaCl concentrations. Total number of 10 strains isolated in which one was fungus and rest bacterial both gram's positive and gram's negative. The pigmented strains isolated from lakes of Bhopal include *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Staphylococcus aureus*, *Serratia marcescens* and *Rhodotorula mucilaginosa* and non–pigmented *Escherichia coli, Pseudomonas fluorescens*, *Bacillus licheniformis*, *Citrobacter freundii* and *Aspergillus niger*. The results showed that yellow pigment production gradually increased at high temperature, not only yellow, other also increased. The growth and pigmentation increased at high pH especially orange strains and decreased in high NaCl concentrations. The pigments had wide industrial use, biodegradable and non –toxic, further can be used as coloring agent. But may cause opportunistic infections in immunocompromised patients. Therefore, swimmers had to take precaution to protect themselves from infection.

1. Introduction

The aquatic ecosystem like freshwater lakes, ponds and wetlands have diverse group of microflorae due to availability of nutrient, organic matter and heterogeneity of environment [1]. The aquatic environment is exposed to sunlight which support growth of bacteria and pigmentation in bacteria due to photooxidative damage and environmental stress. Lakes and other associated ecosystem are one of the important reservoirs of pigmented microbes [2]. Many pigmented microbes have photosynthetic properties also like cyanobacteria. The production of pigment helps microbes in number of ways like protection, physiological and ecological functions, oxidative stress, defense against other pathogenic microbes, resistance to ultraviolet radiation and to adapt in environmental stress [3].

The microbial pigments include Quinone's, Flavin's, prodigiosins, violacein, etc. [4-6]. The color of these pigments produced may vary from red, orange, yellow, violet, blue, green or brown depending on environmental conditions and their chemical composition [7]. Some pigments produced by microbes are non –toxic and used commercially in cosmetics, medicine, foodstuff, industrial purposes, dying industry, etc. Microbial pigments classified on the basis of chemical structure. The commonest pigment produced by microbes is carotenoid gives red, orange and yellow color which protect bacteria cells from UV light and oxidative stress [8]. Common carotenoid-producing bacteria include *Micrococcus luteus*, *Flavobacterium* and *Rhodococcus.* The pyocyanin produced by *Pseudomonas aeruginosa* is Phenazine pigment involved in electron transport chain and microbial competition [1]. The *Chromobacterium violaceum* produces violet color pigment called Violacein which exhibits anticancer and antimicrobial activities [9]. A red pigment prodigiosin produced by *Serratia marcescens* have been reported to possesses immunosuppressive, antimicrobial, and anticancer activities [10]. Violacein, purple or violet colored pigment produced by *Chromobacterium violaceum* possesses antibacterial, antiviral, antitumor, and antioxidant properties [9]. Microbial pigments have some beneficial properties like antiproliferative, antibiotic, antioxidant, immunosuppressive, anticancer, etc. [11,12].

In the present study pigment producing bacteria were isolated from lakes of Bhopal. The "city of lakes" Bhopal, capital of Madhya Pradesh has number of artificial lakes and popularly called "city of lakes." The lakes include Upper Lake, Lower Lake, Shahpura Lake, and Kaliyasot Reservoir. These lakes support diverse aquatic ecosystems and receive nutrients from natural and anthropogenic sources. Variations in physicochemical parameters such as pH, temperature, dissolved oxygen, and organic matter content create favorable conditions for microbial growth and diversity. Therefore, the lakes and surrounding ecosystems of Bhopal may serve as suitable habitats for pigment-producing bacteria. Urbanization, sewage discharge, agricultural runoff, and anthropogenic activities have altered the ecological characteristics of many lakes in Bhopal. Such environmental variations may influence microbial population dynamics and promote the growth of stress-tolerant pigment-producing bacteria [13]. The present study aims to isolate, characterize, and analyze pigment-producing bacteria from selected lakes and surrounding ecosystems of Bhopal. The study may contribute to understanding microbial diversity and exploring bacterial pigments with potential industrial and biomedical applications. The findings of this study may contribute to environmental microbiology, microbial biotechnology, and natural pigment research.

2. Experimental Methods

The present study was carried out using freshwater samples collected from different lakes and surrounding area of Bhopal. Lakes in Bhopal are subjected to varying environmental conditions including nutrient fluctuations, anthropogenic activities, sunlight exposure, and seasonal changes that may influence microbial diversity and pigment production. The samples were collected during the month of April 2026 from Upper Lake near Karbala, Van Vihar, Boat Club, Mandir area upper lake, Badhbada Dam area, Shahpura lake, Chota talab Setu Bridge, Chota talab near Mandir, Chota talab near Kamla Park, Sarangpani Lake, BHEL area, Motia Talab, Saryu Sarovar Park Lake near Ayodhya Bypass, Hataikheda Dam, Bhopal Lake near Sheetal Daas ki Bagiya and Khatlapura lake point.

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2.1 Sample Collection

Water and sediment samples were collected aseptically from lakes sites and surrounding areas using sterile swab sticks (Fig. 1). Precautions during sample collection included use of sterile swab sticks, avoidance of external contamination, proper labeling of samples, immediate transportation to laboratory. The collected samples were transported to laboratory and processed within 24 hours of collection. Environmental observations such as the following were also recorded sampling location, date of collection, temperature, pH, water color and visible environmental conditions.

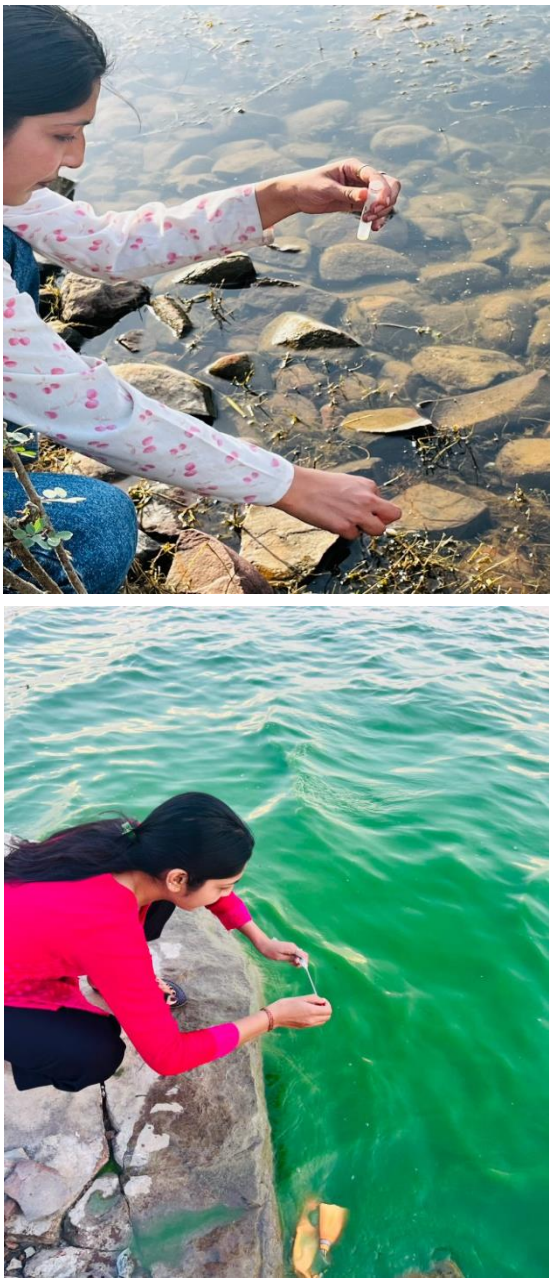

Fig. 1 Sample Collection from upper and lower lake of Bhopal

2.2 Isolation of Microflora by Culture Method

The swab samples collected from different lakes was inoculated on culture plates of Nutrient Agar media. After inoculation plates were incubated at 37 °C for 24 hrs. After 24 hours of incubation, plates were examined for colony morphology, color and texture. The colonies were further identified by gram's staining and biochemical tests. Morphological characteristics of bacterial isolates were recorded based on colony appearance and microscopic observations. The following parameters were recorded and colony characterization include color, shape, margin, elevation, surface texture, opacity, and size. The microscopic examination of bacterial cells for cell shape, cell arrangement, motility and gram staining results. After gram's staining and identified strains were sub-cultured in nutrient broth for pure culture. The pure culture bacteria were further used for biochemical analysis.

2.3 Biochemical Tests

2.3.1 Catalase Test

The test organisms were identified by picking up bacterial colonies on a clean slide in distilled water and to it 1 – 2 drops of hydrogen peroxide (H_2O_2) added, covered with the plate. Presence of gas bubbles indicates positive result due to catalysis of hydrogen peroxide by bacterial enzyme catalase in water and oxygen. The tests differentiate *Staphylococcus* species which gives positive result from *Streptococcus* gives negative result. Fresh 24-hour culture samples to be taken and H_2O_2 solution kept in black bottle and shaken before use.

2.3.2 Coagulase Test

The presence of enzyme coagulase converts fibrinogen to fibrin and causes clotting of plasma in *Staphylococcus aureus.* On a clean slide two drops of saline taken and colonies emulsified and thick suspension made. A loopful of undiluted human plasma mixed with bacterial suspension, an immediate coarse clumping of mixture in 5 – 10 seconds examined for positive results. One negative control to compare with test in which no plasma added for no coagulase clumping. The *Staphylococcus aureus* gives positive result and *Staphylococcus epidermidis* gives negative result.

2.3.3 Triple Sugar Iron (TSI) Test

Some microbes have ability to ferment sugars, gas production and changes color of the pH indicator from red to yellow. Some bacteria ferment sugars and produce hydrogen sulfide and turns media black. The TSI media was prepare 500 mL and poured in sterile test tubes with cotton plug. After pouring, tubes are kept in tilting position for slant preparation and after solidification bacteria inoculated using sterile loop by streaking as well as stabbing in media center. The tubes were incubated at 37 °C for 18 to 24 hours after inoculation. The medium reaction with color changes observed after 24 hours of incubation. The color changes include red slant/yellow butt for an alkaline or acid indicates dextrose fermentation only. The yellow slant/yellow butt for an acid/acid indicates dextrose, lactose and/or sucrose fermentation. The red slant/red butt for alkaline/alkaline reaction indicates absence of carbohydrate fermentation. Blackening of medium occurs in the presence of H_2S. The production of gas CO_2 and H_2S produces bubbles or cracks in the agar. The tests differentiate gram negative rods from other members of the Enterobacteriaceae family on the basis of sugar fermentation.

2.3.4 Hemolysis Test

Some bacteria cause hemolysis of blood and identified by this test. Blood agar plates used for bacterial growth that are capable to produce extracellular enzymes which causes blood hemolysis. The blood also aids in visualizing hemolytic reactions of different bacteria. The medium contains peptone, tryptose for carbon, nitrogen, amino acids, vitamins and minerals and sodium chloride to maintain osmotic equilibrium. Sheep blood mostly used for Group A *Streptococci* and provides best results because it is efficient in pyridine nucleotides. In the present study Sheep blood agar prepared plates were procured from Himedia were used for the study. The pure culture strains were inoculated onto plates and plates were incubated at 37 °C for 24 hours. The plates were examined after 24 hours for hemolysis and bacterial identification. Some bacteria like *Streptococcus pneumonia* causes partial hemolysis with greenish grey or brownish coloration around colony called alpha hemolysis. Some causes complete hemolysis of blood by clear zone around colonies called beta hemolysis like *Streptococcus pyogenes*. Some bacteria are non –hemolytic causes no lysis of red blood cells called gamma hemolysis.

2.3.5 Citrate Utilization Test

This biochemical test helps for identification of enterobacteria which utilize citrate as a sole source of carbon and metabolise to acetoin and CO_2, as well as ammonium as its only source of nitrogen. In the present study Simmon's Citrate agar was used procured from Himedia. The media was weighed and 500 mL prepared. The media was autoclaved and poured in test tubes for stab culture. Allow it to solidified and sample inoculated using loop by stab method. A growth of blue color in Simmon's agar, indicates positive result with citrate utilization. The *E. coli* gives negative result and *Klebsiella pneumonia* gives positive result.

2.3.6 Urease Test

Some bacteria possess urease enzymes which decompose urea by hydrolysis to give ammonia and carbon dioxide, differentiates enterobacteria. The reaction turns medium alkaline which is shown by color change to red pink. Christensen's basal medium prepared, pH

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maintained to 6.8 – 6.9 and autoclaved at 121 ℃ for 20 minutes and to it sterile glucose and urea solutions added by filtration. The medium dispensed in sterile tubes and allowed to solidified. After solidification bacteria inoculated and incubated at 37 °C in incubator overnight. Observe the red – pink color of urease positive test. The *Proteus vulgaris* gives positive result and *Escherichia coli* gives negative.

2.3.7 Methyl Red Test

Methyl red is an azo dye which detect high acidic environment due to pH change as indicator. The test is used to differentiate enterobacteria during glucose fermentation. MR – VP broth (a glucose supplemented peptone medium) prepared and poured in test tubes. The tubes autoclaved and bacteria inoculated in medium. The tubes are incubated at 37 °C for 48 hours up to 5 days to allow sufficient acid to be produced. A few drops of the Methyl Red indicator solution are added to the incubated broth. A red color indicates a positive (acid) reaction. The positive control is *E. coli* and negative control is *Enterobacter aerogens*.

2.3.8 Voges – Proskauer Test

Some bacteria ferment carbohydrates with acetoin (acetyl methyl carbinol) production under alkaline condition, the acetoin is oxidised to diacetyl which gives pink color with the reagent. Inoculate about 2 mL of the medium with the test organism and incubate at 37 °C for 48 hours. Add 1 mL of 10% potassium hydroxide. Leave at room temperature for one hour. Presence of pink color indicates positive result and no color change is negative, examples are *Escherichia coli* and *Klebsiella pneumonia*.

2.4 Determination of Maximum Pigment Production at Different Parameters

The pure culture bacterial strains further cultured on nutrient broth and effect of different parameters determined. The parameters used are given below. All the results were compared with standard growth.

2.4.1 Temperature Effect on the Pigment Production

The 4 sets of nutrient agar plates were taken inoculated with bacterial strains and incubated at different temperature 25 °C, 30 °C, 35 °C and 40 °C for 24 hours. After incubation growth observed and effect of temperature determined on pigment production [14].

2.4.2 Effect of pH on the Pigment Production

The bacterial pigmented strains were inoculated in nutrient broth with varying pH of 2, 4, 7, and 9. The pH of media adjusted using 1 M NaOH and 1 M HCl. The broth incubated for 24 hours at 37 °C to study pH effect on pigment producing bacteria [14].

2.4.3 Effect of NaCl on the Pigment Production

Nutrient broth prepared using different concentrations of NaCl (0.5%, 1%, 4%, 7%, 10%), respectively and autoclaved. After autoclaving the bacterial suspensions were inoculated in culture medium and incubated at 37 °C for 24 hours. The growth effect was observed and pigmentation was determined [14].

2.5 Statistical Analysis

All experiments were performed in triplicate and results are expressed as mean ± standard deviation (SD). The mean, standard deviation and percentage reduction values were calculated in MS excel.

3. Results and Discussion

In the present study 100 samples were inoculated from Bhopal lakes and surrounding areas showed 10 different types of colonies pigmented and non–pigmented.

The colony morphology examined and color of colonies varies from white, yellow, orange, pink and dark yellow colonies. In one of the samples taken from lower lake apart from bacteria fungal growth also observed black and white.

The details of colony morphology and colony characterization were given in tabular form (Table 1). Most of the colonies obtained from different lakes were non- pigmented or yellow colored. In some of the samples of upper lake pink colonies and orange colonies were noted. The number of colonies counted and was found approximately more than 1000 to 50 colonies and varies in different samples inoculated. The maximum growth was observed in samples taken from lower lake and Shahpura lake. The growth was observed in 24 hours and size of the colonies increased after prolonged incubation (Table 1 and Fig. 2).

Table 1 Bacterial Colony Morphology of different strains isolated from lakes of Bhopal

Color	Size of colony	Shape	Margin	Consistency	Opacity	Elevation
White (small)	1 mm	Circular	Entire	Smooth	Translucent	Flat
White (Big)	10 mm	Irregular	Undulated	Brittle	Opaque	Flat
Yellow	1 mm	Circular	Entire	Smooth	Opaque	Flat
Dark yellow	1 mm	Circular	Entire	Smooth and mucoid	Opaque	Flat
White with slight green	2 mm	Circular	Entire	Smooth and mucoid	Opaque	Flat
Orange	1 mm	Circular	Entire	Smooth	Opaque	Flat
Pink	1 mm	Circular	Entire	Smooth and shiny	Opaque	Flat
Orange red	1 mm	Circular	Entire	Smooth and mucoid	Opaque	Flat
Black fungus	2 -3 mm	Round and irregular	Entire and fringed	Velvety or fluffy	Opaque	Raised
White fungus	2 mm	Round	Entire and fringed	Velvety or fluffy	Opaque	Raised

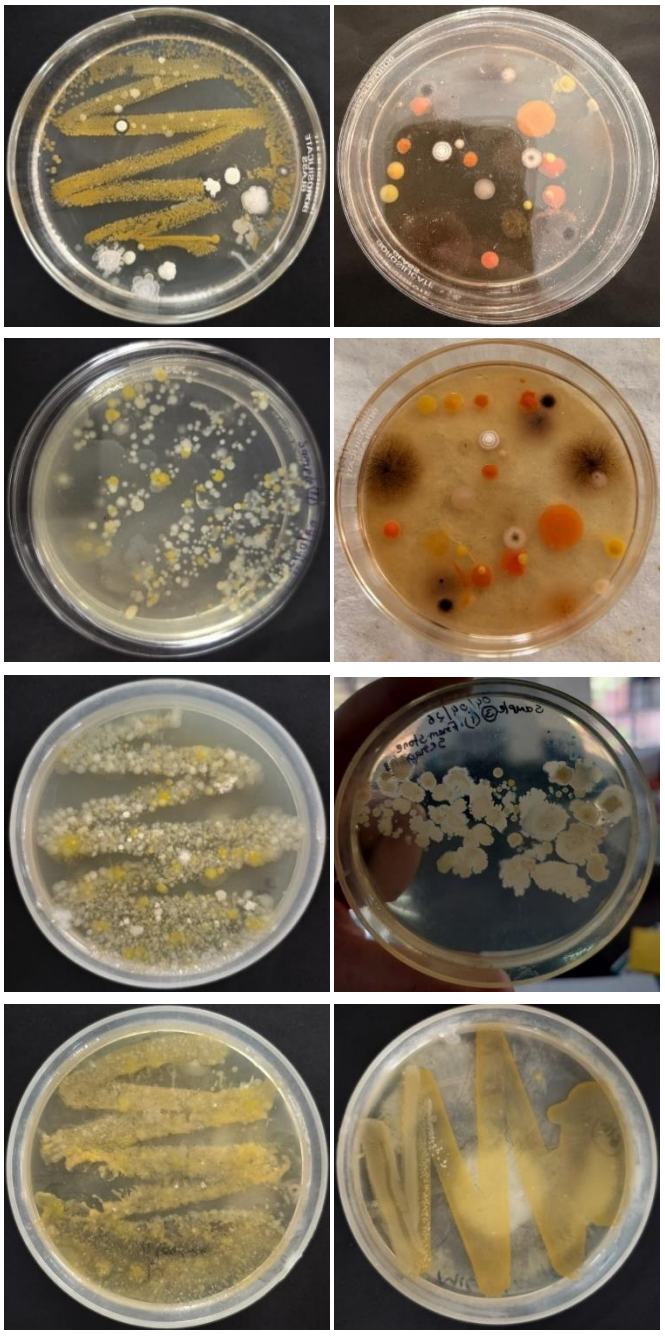

Fig. 2 Nutrient agar culture plates with pigmented and non-pigmented bacterial colonies

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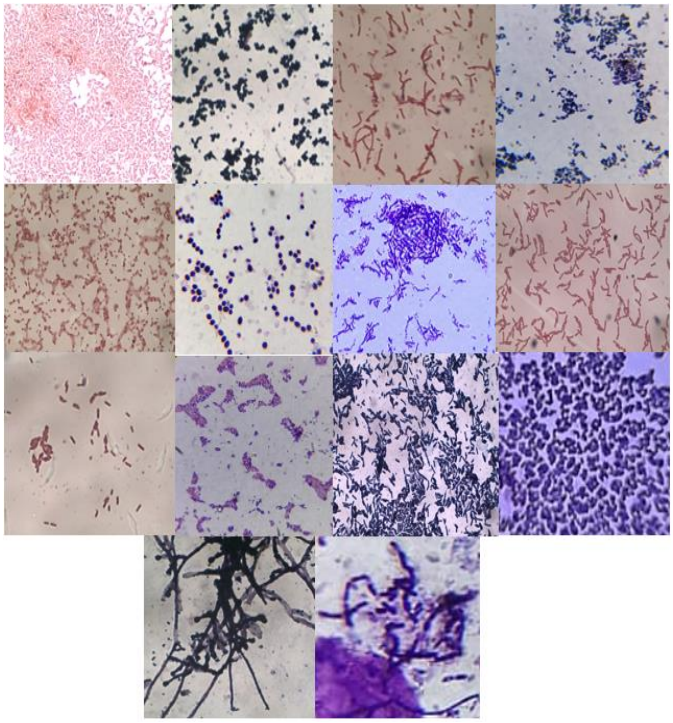

Fig. 3 Bacterial and fungal strains isolated from lakes of Bhopal

Table 2 Identification of bacterial strains by Biochemical tests

Bacterial strains	Catalase	Coagulase	Citrate Utilization	Urease
Escherichia coli	positive	negative	negative	negative
Micrococcus luteus	positive	negative	negative	positive
Staphylococcus aureus	positive	positive	positive	positive
Pseudomonas fluorescens	positive	negative	positive	negative
Pseudomonas aeruginosa	positive	negative	positive	negative
Rhodotorula mucilaginosa	positive	negative	positive	positive
Serratia marcescens	positive	negative	positive	negative
Bacillus licheniformis	positive	negative	positive	positive
Citrobacter freundii	positive	negative	positive	negative
Aspergillus niger	positive	negative	-	positive

Table 3 Identification of bacterial strains by Biochemical tests

Bacterial strains	TSI	MR	VP	Hemolysis
Escherichia coli	gas production with bubbles, yellow slant and butt due to acid production	positive	negative	Beta hemolysis
Micrococcus lutens	Negative with no color change	negative	negative	Gamma hemolysis (No hemolysis)
Staphylococcus aureus	Yellow slant and butt due to acid production	positive	positive	Beta hemolytic
Pseudomonas fluorescens	Negative with no color change	negative	negative	Gamma hemolysis (No hemolysis)
Pseudomonas aeruginosa	Negative with no color change	negative	negative	Beta hemolysis
Rhodotorula mucilaginosa	Negative with no color change	negative	negative	Gamma hemolysis (No hemolysis)
Serratia marcescens	Yellow slant and butt due to acid production. No gas	negative	positive	Gamma hemolysis (No hemolysis)
Bacillus licheniformis	Yellow butt due to acid production	negative	positive	Beta hemolysis
Citrobacter freundii	gas production with bubbles, yellow slant and butt due to acid production. Positive H_2S causing blackening of butt	positive	negative	Gamma hemolysis (No hemolysis)
Aspergillus niger	-	-	-	-

3.1 Biochemical Tests

The results on the basis of biochemical tests showed 4 gram positive, 5 gram negative and 1 fungal strain pigmented and non-pigmented. The bacterial strains isolated was *Escherichia coli*, *Micrococcus lutens*, *Staphylococcus aureus*, *Pseudomonas fluorescens Pseudomonas aeruginosa*, *Rhodotorula mucilaginosa*, *Serratia marcescens*, *Bacillus licheniformis*, *Citrobacter freundii* and one fungus *Aspergillus niger* (Tables 2- 4 and Fig. 3). The maximum bacterial strain isolated was *E. coli.*

Table 4 Bacterial strains isolated and identified by gram staining

Microbial strains	Colony color	Gram stain
Escherichia coli	White	Gram negative rod shaped
Micrococcus luteus	Bright yellow	Gram positive cocci
Staphylococcus aureus	Golden yellow	Gram positive cocci
Pseudomonas fluorescens	White with slight green color	Gram negative bacilli (curved rods)
Pseudomonas aeruginosa	Faint green-yellow	Gram negative bacilli
Citrobacter freundii	Opaque grey	Gram negative
Rhodotorula mucilaginosa	Pink to orange	Gram positive oval budding yeasts
Serratia marcescens	Red	Gram negative bacilli
Bacillus licheniformis	White	Gram positive bacilli
Aspergillus niger	Black	conidia and hyphae

3.2 Pigment Production Efficiency at Different Parameters

The results showed temperature increase leads enhanced growth and increased pigment production. The least pigmentation was observed at 25 °C compared to 30 °C, 35 °C and 40 °C. So, increase in temperature showed an increased in growth of bacteria and its pigment production capacity. The pigment production increased with pH increase and maximum pigment production was observed at pH 9. The orange pigmentation production was not found at pH 6 and yellow pigment production was found minimum. It was observed that pH 7 was found to be optimum for maximum pigment production. The pigment production gradually decreased with increase in salt concentration from 4% to 8%, and no growth observed in 10%. The maximum growth was found in 2% NaCl.

3.3 Discussion

In the present study bacteria isolated from lakes of Bhopal. The lakes water is considered fresh as compared to sea and other water bodies due to less salt concentrations. The freshwater ecosystems are rich in micro-organisms as they play an important role as decomposers by breaking down organic matter in nitrate, phosphate and other essential nutrients. Some photosynthetic and pigment producing bacteria are also found due to sun light penetration as they introduce oxygen and energy such as cyanobacteria [15]. But their diversity and abundance can drastically change freshwater quality. There are number of micro-organisms isolated from lakes, in one of the studies from Virginia bacterial strain *Pseudomonas syringae* collected from freshwater surface at a temperature of −8 °C [16]. The pigments produced by bacteria of different colors protect them from injuries, ultra violet and light rays [17]. Some pigments are non–toxic and harmless and have industrial applications, as well as biodegradable in nature [18, 19]. Micro-organisms which have the ability to produce pigments in high yields include species of *Monascus, Paecilomyces, Serratia, Cordyceps, Streptomyces* and yellow-red and blue compounds produced by *Penicillium herquei* and *Penicillium atrovenetum, Rhodotorula, Sarcina, Cryptococcus, Monascus purpureus, Phaffia rhodozyma, Bacillus sp., Achromobacter, Yarrowia* and *Phaffia* also produce a large number of pigments [20]. The bacterial pigments isolated till date and reported and have potential applications include violacein, indigoidine, melanin, carotenoids, prodigiosin, and rhodopsin [21].

The city of lakes, Bhopal is known for its beauty and lakes. The lakes present in Bhopal are all man made. The sample for assessment of micro-organisms pigment producing was done by collecting samples from lakes and dam were collected and its surrounding places. The upper lake is one the oldest lake man made. Geographically, the lake lies between latitude 23°13′N to 23°16′N and longitude 77°18′E to 77°22′E, covering a surface area of approximately 31 square kilometers and a catchment area of 361 square kilometers [22]. The Upper Lake is relatively less polluted compared to the Lower Lake and is primarily used for consuming water supply, supporting nearly 40% of Bhopal's urban population [23]. Ecologically, the lake hosts a variety of biodiversity including aquatic plants, plankton, fish species, micro-organisms and several residents. The lake is rich in microflora but due to urbanization and sewage disposal in lake some pathogenic flora enters water and causes harm. The bacterial load changes in different seasons, especially in rainy season due to water

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flow from different areas carrying sewage. Some of the studies done to evaluate and determine microflora and water quality of Bhopal's Upper Lake after the escape of methyl isocyanate gas from the Union Carbide factory in Bhopal, India. The results demonstrated increased levels of coliform to 24000/mL during pre-monsoon and decreased to a minimum (120/mL) in the monsoon time, compared to the WHO's recommended limit of 100/mL for drinking water. As per results water was found dangerous to drink [24].

In the present study commonest bacterial strain isolated was *Escherichia coli,* gram negative, facultative anaerobic, non- sporing, peritrichous flagella or non-motile, rod-shaped bacterium (bacillus) that typically measures 1–3 µm in length and 0.5–0.8 µm in diameter. The primary source of *E. coli* in water bodies is fecal contamination or by sewage and its elevated levels cause gastrointestinal problems if water sample swallowed [25]. In the present study maximum growth was observed in Lower Lake and Shahpura lake. The bacterial strains isolated include *Micrococcus luteus* gram positive, strict aerobe forms bright yellow, smooth, circular colonies with entire edges and slight sticky texture colonies on nutrient agar. *M. lutens* often found in freshwater environments, soil, and human skin, acts as a harmless saprotroph that feeds on organic debris, though it can become an opportunistic pathogen or serve as a protective probiotic depending on the ecosystem's balance. It is known for its high survivability, including resistance to desiccation and UV radiation [26-28]. The high concentrations of *Micrococcus* are often markers of organic enrichment. It also forms biofilm on submerged rocks and aquatic plants [29]. Another common bacterial strain isolated was *Staphylococcus aureus* is a facultative anaerobe, gram positive, cocci shaped, measure approximately 1 µm in diameter, found in clusters grape like or in chains also. The bacteria commonly found on skin and in nasal passages and contaminate recreational lakes by droppings, as well as remain viable for several days [30]. The contaminated water with this bacterium can cause infections to swimmers with cuts, scrapes or hair follicles and also causes skin, eye or ear infections [31].

Another bacterium isolated in some samples was *Pseudomonas fluorescens* is aerobic, gram negative, motile, rod-shaped bacterium (bacillus) that measures 1.5 to 3.0 µm in length and 0.5 µm in width, and is distinct for producing a yellow-green fluorescent pigment called pyoverdine used to scavenge iron from water [32, 33]. *P. fluorescens* is found naturally in soil and freshwater lake ecosystems primarily acts as a beneficial decomposer or agricultural bio-control agent, high concentrations can serve as an opportunistic pathogen by causing skin and eye irritation [34, 35]. The bacteria naturally help degrade environmental pollutants and can act as an antagonist against harmful crop fungi (e.g., *Fusarium* wilt) [36]. *Pseudomonas aeruginosa* is aerobic, gram-negative, rod-shaped bacterium measuring approximately 0.5 to 0.8 µm in width and 1.5 to 3.0 µm in length. It is characterized by its ability to produce water soluble distinct colors, emit characteristic sweet grape like aroma, unipolar motility, and distinct colony variations [37, 7]. The pigment includes blue green pigment called pyocyanin, yellow green pigment, pyoverdin and some strains produces red pigment called pyorubin, and brown or black called Pyomelanin [38]. *P. aeruginosa* is a resilient, opportunistic bacterium that naturally inhabits water, soil, and moist environments. It causes systemic opportunistic infections, skin rashes and otitis externa in swimmer's ear [39]. The bacteria secrete a slimy, protective matrix that allows them to cling to rocks, organic debris and surfaces within the water ecosystem. They survive in low nutrient environment also [40]. *Rhodotorula mucilaginosa* is a one of the common freshwater and saltwater yeasts known for production of distinctive coral red, salmon pink or orange color pigmentation due to presence of carotenoid (like torularhodin). It is symbiotic, saprophytic, harmless and have scavenging role in the environment. The carotenoids and lipids act as vital nutrient source for aquatic organisms and naturally colonizes the skin, gills, and gastrointestinal tracts of fish [41]. It causes opportunistic infections in immunocompromised patients [42].

The reddish or pink color hue appear due to presence of *Serratia marcescens.* It is gram negative, facultative anaerobe, cocco bacilli or bacilli, appear singly or chains, measure 0.5 to 0.8 µm in diameter and 1.0 to 2.0 µm in length, possess peritrichous flagella in some strains belonging to the Enterobacteriaceae family. It is naturally found in soil, water, and plant surfaces, while widely distributed in the environment, its presence in lakes typically indicates organic nutrient enrichment or agricultural runoff and known for producing a distinct red pigment called prodigiosin. It causes opportunistic infections in humans which are urinary tract infections, wound infections, and pneumonia [43].

Bacillus licheniformis is facultative anaerobe, gram-positive, rod-shaped bacterium, measure 0.6-0.8 µm wide and 1.5-3.0 µm long, and often forming chains. It is highly motile and features unique spore-forming characteristics, widely distributed in soil, water, and on aquatic plants. In lakes, it plays a vital role in organic matter decomposition, nutrient cycling, and water quality regulation, while also serving as a natural probiotic that supports the immune health of aquatic species [44]. The bacteria secrete extracellular enzymes like amylases and proteases to break down complex organic matter, dead vegetation, and waste. This speeds up nutrient recycling, making vital carbon, nitrogen, and phosphorus available to other aquatic life [45] and lowers harmful compounds such as ammonia, nitrites, and excess phosphates by regulating pH and maintain a healthy microbial balance in the aquatic environment [46].

Citrobacter freundii is a facultative anaerobic, gram-negative rod, found natuarally in soil, water and GI tract of humans and animals, measure 1.0 µm in width by 2.0--6.0 µm in length, peritrichous flagella belonging to the *Enterobacteriaceae* family. Its high concentrations lead opportunistic infections like urinary tract infections (UTIs), diarrhea, respiratory infections, and bacteremia (bloodstream infections) [47]. *Aspergillus niger* is a common filamentous fungus, identified by its distinct macroscopic and microscopic features dense, cottony white or yellow mycelial colony that rapidly matures into a powdery, jet-black surface due to heavy conidial (spore) production. It grows on soil or dead or decay and enter water bodies causes opportunistic infections otomycosis (swimmer's ear) or fungal keratitis. Interestingly, *A. niger* plays a vital role in lake water cleanup by biodegrading industrial effluents and biosorbent to remove heavy metals and microplastics [48].

4. Conclusion

In the present study pigment and non–pigment producing bacteria were isolated from lakes and surrounding areas of Bhopal. The pigmented strains isolated from lakes of Bhopal include *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Staphylococcus aureus*, *Serratia marcescens* and *Rhodotorula mucilaginosa.* These bacteria produce pigment and turns media color. The pigments present in bacteria provides protections against harmful products and have wide industrial applications. These pigmented strains are not harmful for our lakes also. But may cause opportunistic infections in immunocompromised patients. Therefore, swimmers had to take precaution to protect themselves from infection. Further these pigments can be isolated for industrial uses as pigments have antimicrobial activity also.

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