CHAPTER · VIII

SUMMARY AND CONCLUSION
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The present investigation entitled "Isolation, Identification and Characterization of Bacterial Cellulases from Sagar Lake", was carried out during the study period. Sagar lake supports diverse kinds of planktonic flora and fauna which is conditioned to several fluctuating seasonal, environmental and biological factors. Although the biota present is natural but at times it appears that the gradually increasing intensities of outside factors like entry of sewage, hospital waste, opening of drainage, washing, wallowing of cattle, culture of fish and hydrophytes, considerably modify the natural condition of the habitat. This suggests that the physico-chemical parameters and biological properties have undergone a change thus altering the biological conditions leading to eutrophication.

Position in the Map of India, Sagar district placed almost in the central part of the central India. The area lies between the latitude 23°10' to 24°27'N and the longitude of 87°4' to 79°21'E. Geologically the rock formation of the district classified in two groups i.e., (i) the Deccan Trap basalt and (ii) the Vindhyan sandstone. Sagar city has a pleasant climate. On the basis of rainfall, temperature and relative humidity the climate can be divided into three distinct seasons, namely rainy, winter and summer. Sagar lake is situated in the centre part of the Sagar city and it covers a area of 82 hectares.
Three stations from the Sagar lake have been selected for the present study.

1. Chakra Ghat (CG)  SITE – I
2. Temple Site (TS)  SITE – II
3. Dhobi Ghat (DG)  SITE – III

In all the above sites, detergents are used by households for washing cloths, cleaning utensils and bathing. Sewage contains food residues, domestic wastes, soap, detergent etc. normally pollute the water.

PHYSICO-CHEMICAL STUDIES

The physico-chemical properties of SITE – I (Chakra Ghat) show the ranges of pH from 8.4 to 8.9, temperature 19°C to 26°C, chloride 60.29 mg/L⁻¹ to 96.29 mg/L⁻¹, alkalinity 7.2 mg/L⁻¹ to 23.2 mg/L⁻¹, total hardness 129.3 mg/L⁻¹ to 167.3 mg/L⁻¹, calcium hardness (CaCO₃) 43.26 mg/L⁻¹ to 78.86 mg/L⁻¹, calcium hardness (Ca) 17.32 mg/L⁻¹ to 38.78 mg/L⁻¹, magnesium content 14.27 mg/L⁻¹ to 30.14 mg/L⁻¹, CO₂ was absent expect for month of October where it was 2.0 mg/L⁻¹, DO 1.6 mg/L⁻¹ to 2.9 mg/L⁻¹, BOD 11.0 mg/L⁻¹ to 21.3 mg/L⁻¹, COD 21.10 mg/L⁻¹ to 48.1 mg/L⁻¹, phosphate 0.03 mg/L⁻¹ to 0.29 mg/L⁻¹ and nitrate 3.10 mg/L⁻¹ to 10.1 mg/L⁻¹.

The physico-chemical properties of SITE – II (Temple site) showed pH which ranged from 7.1 to 8.7, temperature from 20°C to 26°C, chloride from 68.29 mg/L⁻¹ to 141.99 mg/L⁻¹, alkalinity 12.0 mg/L⁻¹
to 48.6 mg/L\(^{-1}\), total hardness from 120.0 mg/L\(^{-1}\) to 172.2 mg/L\(^{-1}\), calcium hardness as CaCO\(_3\) and as Ca from 63.0 mg/L\(^{-1}\) to 86.73 mg/L\(^{-1}\) and 25.23 mg/L\(^{-1}\) to 34.73 mg/L\(^{-1}\) respectively, magnesium content 9.97 mg/L\(^{-1}\) to 26.32 mg/L\(^{-1}\), free CO\(_2\) (7.2 mg/L\(^{-1}\)) was observed only in the month of October, DO ranged from 1.2 mg/L\(^{-1}\) to 2.7 mg/L\(^{-1}\), BOD 11.2 mg/L\(^{-1}\) to 22.1 mg/L\(^{-1}\), COD 21.2 mg/L\(^{-1}\) to 51.6 mg/L\(^{-1}\), phosphate 0.03 mg/L\(^{-1}\) to 0.24 mg/L\(^{-1}\) and nitrate ranged from 4.8 mg/L\(^{-1}\) to 8.2 mg/L\(^{-1}\).

The physico-chemical studies of SITE – III (Dhobi Ghat) shows that the pH ranged from 7.0 to 8.6, temperature from 19\(^{0}\)C to 26\(^{0}\)C, chloride from 110.29 mg/L\(^{-1}\) to 189.99 mg/L\(^{-1}\), alkalinity from 31.2 mg/L\(^{-1}\) to 63.2 mg/L\(^{-1}\), total hardness from 137.30 mg/L\(^{-1}\) to 178.6 mg/L\(^{-1}\), calcium hardness as CaCO\(_3\) from 60.9 mg/L\(^{-1}\) to 89.40 mg/L\(^{-1}\) and as Ca from 24.39 mg/L\(^{-1}\) to 35.82 mg/L\(^{-1}\) respectively, magnesium content ranged from 11.93 mg/L\(^{-1}\) to 24.26 mg/L\(^{-1}\), free CO\(_2\) (1.8 mg/L\(^{-1}\)) was recorded in October only, DO ranged from 1.6 mg/L\(^{-1}\) to 2.4 mg/L\(^{-1}\), BOD from 13.60 mg/L\(^{-1}\) to 23.0 mg/L\(^{-1}\), COD from 20.5 mg/L\(^{-1}\) to 55.20 mg/L\(^{-1}\), phosphate from 0.05 mg/L\(^{-1}\) to 0.35 mg/L\(^{-1}\) and nitrate ranged from 4.3 mg/L\(^{-1}\) to 8.3 mg/L\(^{-1}\).

**BACTERIOLOGICAL STUDIES**

Heterotrophic plate count (CFU x 10\(^{-4}\)) shows maximum number of colonies (286 ± 1) from SITE – I in July. Variation in shape, size and colour of bacterial colony after 24 h of incubation period was also seen. Minimum number of colonies (104 ± 1) were found from SITE – II in the month of November.
From Sagar lake 120 bacteria were isolated from all the three sites. Out of these 120 isolates, 33 showed cellulolytic nature when screened on Paul’s basal agar media. According to dense positive growth on the media, ten isolates were selected for identification and further studies. Out of the 10 bacterial isolates selected, six were gram positive (CG-A8, TS-B2, TS-B6, TS-B9, TS-E1 and DG-C7) while remaining four isolates were gram negative (CG-A1, CG-A3, CG-D2 and CG-J4). Three isolates (CG-A1, CG-A3 and CG-J4) were rod shaped while seven isolates (CG-A8, TS-B2, TS-B6, TS-B9, DG-C7, CG-D2 and TS-E1) were coccus in shape.

Biochemical tests (indole, methyl red, Voges Proskauer, catalase, citrate, nitrate reduction, oxidase, hydrogen sulphide, TSI, amylase, gelatinase, urease, malonate, gluconate and carbohydrate fermentation) were also performed to identify the 10 selected bacterial isolates. Bergey’s Manual of Determinative Bacteriology (1994) and PIB computer kit (Bryant, 1989) was also used for identification of bacteria. It was found that out of the ten selected bacteria 5 belonged to Micrococcus, (i) Micrococcus varians (CG-A8), (ii) Micrococcus lylae (TS-B9), (iii) Micrococcus species (TS-B9), (iv) Micrococcus luteus-2 (DG-C7), (v) Micrococcus agilis (TS-E1) while the other five were (vi) Vibrio metshnikovii (CG-A1), (vii) Providencia pseudomallei (CG-A3), (viii) Planococcus species (TS-B2), (ix) Neisseria species (CG-D2) and (x) Chromobacterium species (CG-J4).
ENZYMATIC STUDIES

Out of 120 bacterial isolates 33 were screened positive for cellulolytic nature on Paul's basal agar medium. 10 out of 33 isolates showed dense positive growth, hence, these 10 isolates were further used for enzymatic studies. Out of these ten isolates eight were found mesophilic namely, *Vibrio metschnikovii* (CG-A1), *Providencia pseudomallei* (CG-A3), *Micrococcus varians* (CG-A8), *Micrococcus lylae* (TS-B6), *Micrococcus species* (TS-B9), *Neisseria species* (CG-D2), *Micrococcus agilis* (TS-E1) and *Chromobacterium species* (CG-J4) while 2 were thermotolerant namely, *Planococcus species* (TS-B2) and *Micrococcus luteus-2* (DG-C7). All the above isolates produced a complete set of cellulase complex, which is essential for the rapid hydrolysis of cellulosic material.

*Vibrio metschnikovii* (CG-A1) produced highest amount of exo β-glucanase (0.51 IU/mL) when compared to other ten bacterial isolates, while minimum enzyme production (0.04 IU/mL) was recorded with *Providencia pseudomallei* (CG-A3). Maximum endo β-glucanase production (1.20 IU/mL) was recorded with *Chromobacterium species* (CG-J4) while minimum endo β-glucanase production (0.22 IU/mL) was recorded in *Providencia pseudomallei* (CG-A3). Maximum β-glucosidase enzyme production (0.60 IU/mL) was found in *Vibrio metschnikovii* (CG-A1) while minimum enzyme production (0.04 IU/mL) was recorded in *Micrococcus agilis* (TS-E1).
On the basis of screening and enzyme production results, three best cellulolytic bacterial isolates, i.e. *Vibrio metschnikovii* (CG-A1), *Micrococcus luteus*-2 (DG-C7) and *Chromobacterium species* (CG-J4) were selected further to study the effect of various cultural factors like pH, temperature, metals and inhibitors on cellulase enzyme activity. Normal enzyme assays were done at pH 4.8, but the effect of different pH values (4 to 8) indicates that *Vibrio metschnikovii* (CG-A1) and *Chromobacterium species* (CG-J4) showed maximum exo β-glucanase activity at pH 7.0 while *Micrococcus luteus*-2 (DG-C7) showed maximum enzyme activity at pH 8.0. Thus the cellulase of this isolate was found alkaline in nature. The same results were recorded for endo β-glucanase and β-glucosidase, *Vibrio metschnikovii* (CG-A1) and *Chromobacterium species* (CG-J4) which showed its stability at pH 7.0 for exo β-glucanase, endo β-glucanase while these isolates were stable at pH 8.0 for β-glucosidase activity. *Micrococcus luteus*-2 (DG-C7) showed enzyme stability at pH 8.0 for exo β-glucanase, endo β-glucanase and β-glucosidase.

With regard to temperature *Vibrio metschnikovii* (CG-A1) and *Chromobacterium species* (CG-J4) showed maximum exo β-glucanase, endo β-glucanase and β-glucosidase enzyme activity at 45°C while *Micrococcus luteus*-2 (DG-C7) showed maximum activity at 55°C. *Vibrio metschnikovii* (CG-A1) and *Chromobacterium species* (CG-J4) were thermostable when kept at different incubation time (30, 50 and 90 min) at 45°C while *Micrococcus luteus*-2 (DG-C7) was found stable at 55°C, hence, it also shows its thermostolerant nature.
The effect of different metals (CaCl₂, BaCl₂, CuSO₄, K₂HPO₄ and MgSO₄) on the exo β-glucanase, endo β-glucanase and β-glucosidase at 1 mM and 5 mM concentration showed that CuSO₄ when compared to other metals found to be the best for all the three isolates, Vibrio metschnikovii (CG-A1), Micrococcus luteus-2 (DG-C7) and Chromobacterium species (CG-J4) as it increased the enzyme production.

HgCl₂, EDTA and NaN₃ inhibited the exo β-glucanase, endo β-glucanase and β-glucosidase activity of Vibrio metschnikovii (CG-A1), Micrococcus luteus-2 (DG-C7) and Chromobacterium species (CG-J4).

BIODEGRADATION STUDIES

From the results it can be concluded that all the three bacterial strains were capable of removing protein, carbohydrate, COD and BOD. Maximum removal of protein and carbohydrate was brought about by Chromobacterium species (CG-J4). Vibrio metschnikovii (CG-A1) removed maximum COD while maximum BOD was removed by Micrococcus luteus-2 (DG-C7). These strains can be further used for the treatment of waste water.

CONCLUSIONS

From the present studies the following conclusions can be drawn:

- The pH of water of all the three study sites from Sagar lake were found to be alkaline and this may be due to abundance of buffering substances present in the lake water.
The high value of chloride, hardness, DO, BOD, COD and nitrate recorded from all the three study sites indicated that the water is highly polluted.

The physical and chemical characters of water are normally changed due to organic, inorganic compounds and addition of microorganisms to water. The high heterotrophic plate count of the present study also supported this view that the lake water is highly polluted and such changes in lake water are harmful for human health and this also reduces the usefulness of water.

Although 120 bacteria were isolated from Sagar lake, 33 showed cellulolytic nature, out of which 10 were found highly cellulolytic and were used for further studies.

These 10 isolates were identified and out of which 5 belonged to Micrococcus, (i) Micrococcus varians (CG-A8), (ii) Micrococcus lylae (TS-B6), (iii) Micrococcus species (TS-B9), (iv) Micrococcus luteus-2 (DG-C7), (v) Micrococcus agilis (TS-E1) while the other five were (vi) Vibrio metschnikovii (CG-A1), (vii) Providencia pseudomallei (CG-A3), (viii) Planococcus species (TS-B2), (ix) Neisseria species (CG-D2) and (x) Chromobacterium species (CG-J4).

In comparison to other isolates Vibrio metschnikovii (CG-A1) produced maximum exo β-glucanase, endo β-glucanase and β-glucosidase enzyme which can be useful in textile, detergent and food industries.
The stability of exo β-glucanase, endo β-glucanase and β-glucosidase enzyme for Vibrio metschnikovii (CG-A1) and Chromobacterium species (CG-J4) were at pH 7.0 while for Micrococcus luteus-2 (DG-C7) the stability was at 8.0 thus producing alkaline cellulase enzyme hence may be used as laundry detergents additive.

Micrococcus luteus-2 (DG-C7) was found to be thermotolerant in nature as it was active and stable at 55°C. This isolate may be used in paper industry for pulping and bleaching.

All the three selected bacterial isolates, Vibrio metschnikovii (CG-A1), Micrococcus luteus-2 (DG-C7) and Chromobacterium species (CG-J4) were found capable of degrading organic matter. Amongst these, Chromobacterium species (CG-J4) has higher degrading potential which may be used further in waste water treatment.

In lake water, pollution is one of the major problem. The application of cellulase producing microorganisms helps in the treatment of live stock feeds, garbage, factory effluents, agricultural residues and hospital wastes. Microorganisms present in this system slowly degrade organic wastes present in lake water. Cellulases are used in textile, leather, paper and printing ink industries, therefore the highly cellulolytic bacteria can be used further to obtain stable enzyme preparations needed for the conversion of cellulose to glucose.
In lakes and ponds *Eichhornia* (water hyacinth) is one of the major cause for eutrophication and microorganisms (specially bacteria) present in the system can slowly degrade such aquatic plants. Such studies have helped in building the library of important cellulose degrading indigenous bacteria in the institution for further use.