4.0 Conclusions

The salient observations made in the present study lead to the following conclusions:

1. Isolation of 28 haloalkalitolerant isolates from various ecological niches capable of growth on nitrogen free medium.

2. The pH of all samples from different sites was in the range of 8.4 to 10.5 at 30°C and salinity in terms of EC was in the range of 4.8 to 5.9 dSm⁻¹.

3. The ESP value of soil samples ranging from 13.84 to 21.73%, Na⁺ from 10.2 to 15.5 mgL⁻¹, Ca²⁺ from 35.0 to 55.2 mgL⁻¹ and Mg²⁺ from 2.8 to 8.2 mgL⁻¹. The SAR value of each soil sample was between 2.5 to 3%.

4. Most soil samples were found to be marginally carbon rich (3-7%) and nitrogen deficient (120-175 kg ha⁻¹).

5. The total population of bacteria ranged from $5 \times 10^8$ - $15 \times 10^8$ CFU g⁻¹ of soil, while, fungi and actinomycetes ranged from $2 \times 10^3$ - $6 \times 10^3$ and $5 \times 10^3$ - $15 \times 10^3$ CFU g⁻¹ of soil, respectively.

6. All 28 isolates appeared to tolerate 1M NaCl concentration. Of these, 14 isolates were found to be tolerant to both NaCl concentration (1M) and wide pH (7 – 11).

7. LGI-BTB assay showed blue coloured zones with varying diameter ranging from 2 – 14 mm for all the 14 strains, suggesting diazotrophic activity under alkaline saline stressed conditions.

8. The ethylene productivity by ARA varied among isolates from Lonar Lake (RAL1, RAL6 and RAL9) and mangrove region (RAP1, RAP2, RAP3, RAP4 and RAP5).
9. All isolates from mangrove region were found to reduce acetylene to ethylene. Isolate RAP 3 was found to produce 23.1 nmole L\(^{-1}\) ethylene in 12 days of incubation followed by RAL6 8.34 nmoles L\(^{-1}\) ethylene over a period of 12 days in NF-LGI medium.

10. The isolate RAP3 was identified as *Halomonas venusta* and RAL6 was identified as *Rheinheimera* spp. using morphological, biochemical, physiological, FAME and 16S rRNA sequencing. The gene sequences of both strains submitted to NCBI Genbank database (accession number: JQ229775 and JQ229786 for *H. venusta* RAP3 and *Rheinheimera* spp. RAL6, respectively).

11. The use of degenerate primers successfully amplified and demonstrated the presence of *nifH* gene in *H. venusta* RAP3. To our knowledge, the present study may be the first report for demonstration of the presence of nitrogenase (*nifH*) gene in *H. venusta*.

12. Optimal growth and nitrogen fixation measured using micro-Kjeldahl method among both strains grown in LGI medium containing 1M NaCl under static condition, at 30\(^\circ\)C pH range 7.5 – 8.5 suggesting alkaliphilic nature.

13. Both strains grew maximally at 0.8M NaCl and nitrogen was fixed to nearly 30-31 mg N L\(^{-1}\).

14. The inoculum size of 5% was found to be adequate for maximum growth and yielded 32.96 mg N L\(^{-1}\) in case of *H. venusta* (RAP3) and 31.30 mg N L\(^{-1}\) with *Rheinheimera* spp. (RAL6) after 48 h.

15. Both strains demonstrated PGP activity apart from diazotrophic activity under alkaline-saline conditions. IAA production by both strains in NF-LGI medium under salt and alkali stress was 0.040 gL\(^{-1}\) for *H. venusta* (RAP3) and 0.038 gL\(^{-1}\) for *Rheinheimera* spp. (RAL6).
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16. ACC deaminase activity for *H. venusta* (RAP3) was 32.01 nmoles mg\(^{-1}\) and 29.0 nmoles mg\(^{-1}\) for *Rheinheimera* spp. (RAL6).

17. Ammonia, HCN and siderophore were produced by both the strains, but unable to perform phosphate solubilization under stressed conditions.

18. Isolate RAP3 and RAL6 showed reduction in surface tension from 72 mN m\(^{-1}\) to 43 mN m\(^{-1}\) and 42 mN m\(^{-1}\), respectively and EPS production of 1.95 viscosity/ mPas for RAP3 and 2.56 viscosity/ mPas for RAL6.

19. Both strains were resistant to streptomycin (100 µg), indicating rhizospheric origin.

20. Wheat seedling emergence experiment indicated seedling growth was vigorous, rapid and stimulated in the presence of isolates under alkali-saline conditions, but untreated seeds failed to germinate even after 3 days.

21. Pot assay revealed vigour index of *H. venusta* (RAP3) of 18.59, 29.66 and 51.09 % after 30, 60, 90 days. *Rheinheimera* spp. (RAL6) after 30, 60 and 90 days showed 20.66, 30.1, and 52.26 % and after 30, 60 and 90 days RAP3 + RAL6 treatment showed 19.9, 30.83 and 53.83 %. But gypsum treated and *Azotobacter* spp. (positive control) augmented assay revealed negligible vigour index.

22. Exchangeable Na\(^+\) ion concentration of soil sample during pot assay with wheat before and after treatment recorded removal of 35-38% of Na\(^+\) ions by *H. venusta* RAP3, *Rheinheimera* spp. RAL6 and combination of RAP3 and RAL6 while *Azotobacter* spp. (positive control) and gypsum treatment was able to remove only 12% of Na\(^+\).

The rigorous screening of cultures at various levels finally led to selection of two bacterial isolates with diazotrophic and PGP activities at pH 9.5 and 1M NaCl that were evaluated in the pot experiment using alkaline saline soil. The systematic approach
adopted from selection of ecological niches to growth promotion in alkaline-saline soil under greenhouse condition has led to more realistic selection of robust moderately halotolerant alkaliphilic bacteria for wheat plant growth.

**Future studies**

The realistic assessment of bacterial isolates from the present study can be analyzed at field scale level. Further, rigorous analysis with respect to removal of soluble salts may unravel the real bioremediation capabilities of isolates.