EXPLORATION OF HALOTOLERANT ACTINOMYCETES FROM SALT AFFECTED SOIL AND THEIR UTILIZATION TO AMELIORATE SALINITY STRESS IN WHEAT (*Triticum aestivum* L.)

SUMMARY
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Summary

Soil salinity is a worldwide environmental problem that adversely affects crop productivity as well as quality of produce in arid, semi-arid regions and in coastal areas (Rengasamy 2010). Approximately 20% of the irrigated crop area in the world has been reported to be affected by salinity, and the situation is continuously deteriorating (Hasanuzzaman et al. 2013). According to the FAO Land and Nutrition Management Service (2008), over 6% of the global areas are affected by either salinity or sodicity accounting for more than 800 million ha of land (Yadav et al. 2011). Between the years 2015 and 2050 the global population is expected to increase to ~10 billion. This increase in population, along with rising economics, will exert greater pressure on demands for crop and livestock products. Currently, the growth rates in the yields of maize, rice, wheat, and soybean are insufficient to meet projected food demands by 2050 (Ray et al., 2013). The causes of declining growth rates include increasing pressure from salinity and water logging, depletion of soil nutrients and organic matter, climate change, and inappropriate crop management practices (Wichelns and Qadir, 2015).

The Indo-gangetic plain (IGP) is one of the most extensive fluvial plains of the world. It accounts for the one third cultivable lands and is a major contributor of the food supply for the Indian population (Shrivastava et al. 2015). The Indo-Gangetic plain is characterized by intensive agriculture, largely by resource-poor small and marginal farmers. By the year 2050, the Indo-Gangetic region, India's bread basket, will face intense pressure from uncontrolled population growth, in terms of land and water resources, both of which are vital elements for sustainable agricultural growth (FAO, 2011). During the past five decades, various soil-forming processes such as calcification, leaching, lessivage, salinization and alkalinization, gleization and homogenization have been identified in the IGP (Srivastava et al. 2015). Vast swatches of salt-affected areas in the region provide both challenges and opportunities to bolster food security. Due to the emergence of environmental pollution and health concerns for both producers and consumers by exhaustive use of chemicals for saline soil reclamation, developing salt-tolerant crops has been a

much desired scientific goal in recent times. However it has met with little success to date, as only few major-determinant genetic traits of salt tolerance have been identified (Munns and Tester 2008; Schubert et al. 2009). An alternative strategy to improve crop salt tolerance may be to introduce salt-tolerant microbes that could provide an effective eco-friendly approach to reduce environmental and health problems and enhance crop growth. Since soil microbes have been reported to enhance the growth of many different crops grown in a wide range of root-zone salinities, this approach may succeed where it has proved difficult to develop salt-tolerant lines. Indeed, several recent studies have demonstrated that local adaptation of plants to their environment is driven by genetic differentiation in closely associated microbes (Rodriguez and Redman 2008).

Actinomycetes (actinobacteria) are Gram positive bacteria that have a great capacity to survive in adverse environments such as saline soils and are reported to possess plant growth promoting properties. Among them, halophilic and halotolerant actinobacteria are recently gaining much attentions. Despite this, only few reports are available on actinomycetes addressing the issue of plant growth promotion in saline soil. Metabolites and biological functions from halophilic or halotolerant members of this bacterial group may resolve the ever increasing thirst of industry for metabolites with salt tolerant capacity to combat a range of issues from environmental pollution to disease and hunger (Hamedi et al. 2015). The present study was undertaken with the objectives to isolate and characterize halotolerant actinomycetes from stressed habitats with plant growth promotion potential, proline production capability and their evaluation under pot assay for growth promotion of wheat plants in saline stressed condition.

Saline soil samples were collected from salt affected regions of five districts of IGP including Fatehpur, Kanpur, Auraiya, Etawah and Mainpuri, Uttar Pradesh, India. Pre-treatment of soil samples was done for isolation of actinobacteria and suppression of other unwanted microbes. A total of 55 isolated actinobacteria were screened for their *in vitro* salt tolerance capacity by evaluating their growth on NaCl amended media. 28 halotolerant/halophilic isolates were selected for further study.

The halotolerant strains were evaluated for their plant growth promotion traits *viz.* indole acetic acid (IAA) production, P-solubilisation, siderophore, HCN, chitinase and ammonia production. Biocontrol potential of isolates was tested against two fungal phytopathogens (*M. phaseolina* and *Sclerotium rolfsii*) by dual culture assay as well as interaction study using scanning electron microscopy. The morphological characterisation and biochemical tests (such as amylase, protease, cellulase, urease, gelatinase and H_2S production) were performed for the preliminary identification of the isolates. The special characteristics of the halotolerant isolates were determined by study of their growth at different pH and temperature and physiological characterisation was done by studying carbon utilization profile. The halotolerant isolates were characterised using molecular tool *i.e.* 16S rDNA amplification followed by Restriction Fragment Length Polymorphism (RFLP). Finally, the identity of the isolates was confirmed by 16S rDNA sequencing and the nucleotide sequences of 16S rRNA gene were deposited in NCBI GenBank. PCR amplification for hydrolytic enzyme chitinase (*chiA*) gene was performed using the gene specific PCR primers. For *in vivo* pot assay two isolates K34 and K36 were selected based on their PGP attributes and proline production capacity under stressed conditions. The isolates were analysed for their plant growth promotion ability with wheat in non-saline and saline conditions (soil pH- 9.2; EC- 4.62 dS/m). Wheat seeds were procured from Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The pot assay was performed in natural environmental conditions.

The salient results of this study are summarised below:

Out of 55 actinobacteria isolated from salt affected soil samples, 28 isolates were halotolerant with optimum growth at 8% (w/v), NaCl concentration. Since these 28 isolates exhibited good growth in absence or presence of salt, such isolates were designated as moderately (intermediate) halotolerant. All 28 halotolerant isolates were recorded as Indole Acetic Acid (IAA) producers and in two isolates *viz.* K34 and K36 the quantity of IAA produced was directly proportional to salt concentration up to 6% (w/v) NaCl level. A decline was recorded in IAA production beyond 6% NaCl. A total of 22 isolates recorded as ammonia producer, 12 isolates showed siderophore production, eight isolates phosphate solubilisation, six isolates recorded HCN production and chitinase activity was reported only in two isolates. Biocontrol assay against the selected phytopathogen revealed that only two isolates possess antifungal activity; the isolate K20 against *M. phaseolina* and K17 against *Sclerotium rolfsii*. Both the isolates exhibited chitinase activity which was further substantiated by the amplification of 270bp product specific for *chiA* gene in the isolates. The microscopic (SEM) examination of the antagonistic interactions of strains K20 and K17 revealed these strains were entering the fungal mycelia causing distortions and injuries leading to destruction of the mycelia.

The results of phenotypic characterisation of all the 28 halotolerant actinobacterial isolates showed that all of them had hard embedded colonies and varied in the color of aerial and substrate mycelia; 17 isolates having aerial mycelia with grey spores, four isolates having pink spores, three had creamy aerial spore mass, two with white aerial spore mass, and one each had ivory and ash brown aerial spore mass. Soluble pigment production was reported in five isolates. Spore ornamentation studies revealed that most of the isolates had smooth spores. However four isolates, *viz*., K4, K5, K9 and K24 had spiny spores while one isolate K30 had hairy spore morphology. Four different types of spore chains such as straight, rectiflexibilis, retinaculiperti and spirals were observed in the isolates. All 28 isolates were able to grow at a temperature range of 25-50^{o}C and pH range of 5.0-11.0 revealing their moderately alkali-tolerant nature. Out of 10 different sugars tested for carbon utilization, all the isolates were capable of utilizing a minimum of six different sugars as a carbon source. All the 28 halotolerant isolates were producing at least one of the industrially important enzymes *viz.* amylase, protease, cellulase and gelatinase and based on biochemical observations, these strains could be assigned to the *Streptomyces* genera.

Amplification of 16S rDNA was carried out and a single amplicon of about 1150 bp was found in all the isolates. PCR amplification yielded good amount of PCR products which was sufficient for digestion with different tetracutters like *Taq*I, *Hae*III and *Msp*I. The characterization of these isolates on the basis of ARDRA produced polymorphic patterns with different profiles characterized by 3-4 fragments ranging from 50 bp to 450 bp for different isolates. The enzymes, *Hae*III and *Msp*I were found to be more discriminative compared to *Taq*I. Based on 16S rRNA gene partial sequencing, similarity values of >98% suggested that 26 strains belonged to genus *Streptomyces*, and one each strain to *Actinomycete* and to the rare genera *Actinoalloteichus*.

Quantitative estimation of osmolyte (Proline) synthesis in all the 28 halotolerant isolates revealed that all were producing proline to combat adverse effects of salinity stress and the amount of proline synthesized was increasing with higher salinity with maxima in isolate K36 followed by K34. Based on PGP potential and proline synthesis in stressed condition, *in vivo* study done in wheat with *Streptomyces* isolates K34 and K36 revealed that both the isolates were enhancing the growth parameters in wheat under non-saline and saline conditions, individually however, better results were reported when both the isolates were used together. Combination of both the isolates even increased the grain yield of wheat in saline conditions by 109.4% as compared to control.

The present study was successful in isolating and selecting halotolerant actinobacterial strains from salt-affected regions of IGP, Uttar Pradesh, India having plant growth promotion potential and other important attributes. The results not only identified the isolates with PGP potential but also detected strong correlation between salinity and IAA and proline production in isolates. The diversity and functional potential of actinobacterial strains with phosphate mineralisation ability, plant growth promotion potential and biocontrol properties was obtained in the study. Knowledge generated on biodiversity of *Streptomyces* strains with different PGP potential will be helpful to design strategies to use these strains as bio-inoculants for effective management of salinity affected lands. On the basis of above results, the two halotolerant *Streptomyces* strains could be exploited as consortium for sustainable agriculture practice for bioremediation of saline-sodic soil.

Concisely, present study offers the overview on salt tolerance, plant growth promotory activity and molecular phylogeny of halotolerant actinobacteria isolated from the salt affected soil of IGP, Uttar Pradesh, India. The successive efforts to study the diversity of halotolerant actinobacteria from salt affected soil of IGP, U.P.

India, revealed the inhabitance of diverse actinobacteria in saline soil with predominance of *Streptomyces* genera. This study contributes to our acquaintance of saline soil associated actinobacteria and further augments the array of PGP actinobacterial isolates available for sustainable agricultural practices for alleviating deleterious effects of salinity on crops (with special reference to wheat) and our observations certify the beneficial role of *Streptomyces* isolates for the plant growth promotion under salinity stress. Moreover, there is much that will still need to be done, but the findings of this work will form a strong foundation and may serve as a baseline data for enhancing the growth of plants in salinity compromised soil, for bio-management of such soils using halotolerant plant growth promoting actinobacteria.