Chapter 7
Plant Growth-Promoting Bacterial Life at High Salt Concentrations: Genetic Variability

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Abstract Abiotic stresses are emerging environmental factors limiting agricultural productivity around the world. Among these stresses, salt stress is a serious threat affecting crop production especially in arid and semiarid regions of the world. Development of strategies to ameliorate deleterious effects of salt stress on plants has received considerable attention. In this scenario, the use of salt-tolerant plant growth-promoting microorganisms to enhance salinity resilience in crops is encouraged due to their vital interactions with crop plants. Bacteria are widely used to diminish deleterious impacts of high salinity on crop plants because they possess various direct and indirect plant growth-promoting characteristics. This chapter focuses on the effect of salt stress on plants, plant growth-promoting bacterial survival in saline conditions, and their mechanisms to mitigate salt stress at genetic level.

7.1 Introduction

Salinity is one of the major abiotic stresses which negatively affects crop growth and yields and puts down crop production. The presence of high sodium chloride concentration has been reported to cause reduction in microbial flora in the soil (Ibekwe et al. 2010). Most of the world's plateaus confined to the tropics and Mediterranean regions have potential salinity problems (Cordovilla et al. 1994). It is accounted for the presence of naturally high salt levels, salt accumulation during irrigation, or the application of chemical fertilizers. High salinity owing to its toxic effects inhibits the growth of plants by affecting cellular growth and entry of ions across the root system by slowing down the water uptake of plants. Sodium chloride is the most disparaging salt that affects the growth of plants. Saline habitats are frequently inhabited by an abundance of microbial communities adapted to these ecosystems (Zahran et al. 1992). Halophilic bacteria which flourish in hypersaline habitats may retain their potential to express various types of plant growth-

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promoting activities such as phosphate solubilization, nitrogen fixation, or phyto-hormone production. These PGPRs offer promise as potential biofertilizers for improvement of plant growth under stress conditions by reducing the impact of salinity on plant growth and its productivity. Salt-tolerant bacterial species of *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Enterobacter* have been isolated from salt-affected soil and were found to be efficient plant growth promoters (Gopalkrishnan et al. 2012; Allam et al. 2018; Nakbanpote et al. 2014; Kapoor et al. 2017). It is well drafted that indigenous strains have better potential to multiply under stress conditions as compared to the exotic strains. These facts are important while selecting the microbial inoculants for a specific environment. This chapter emphasizes on the assessment of plant growth-promoting rhizobacteria (PGPR) approaches for the alleviation of salinity stress with a brief overview of adaptation mechanism and genetic variability of salt-tolerant strains facilitating them to grow in saline environments.

7.2 Diversity of Salt-Tolerant Bacteria

Salinity affects the structure and species composition of the rhizospheric communities. Saline environments harbor taxonomically diverse bacterial groups such as *Enterobacter*, *Pseudomonas*, *Vibrio*, and a few Gram-positive bacterial species, e.g., *Bacillus*, *Micrococcus*, and *Salinicoccus*, which exhibit modified physiological and structural characteristics under the prevailing saline conditions (DasSarma and DasSarma 2012). Salt-tolerant bacteria have been isolated from different sources such as salt lakes (Hedi et al. 2009), river water (Tiquia et al. 2007), rhizosphere (Hasnain and Taskeen 1989), root nodules (Gal and Choi 2003), and soil samples (Takashina et al. 1994). Gram-negative bacteria including nodulating bacteria have been reported to colonize the saline soil (Zahran et al. 1992). Nodulating bacteria such as *Rhizobium* have been reported in association with the salt marsh grass (Whiting et al. 1986). Among free-living bacteria, those belonging to genus *Azospirillum*, *Bacillus*, *Enterobacter*, and *Azotobacter* play a crucial role in different stressed conditions (Sahoo et al. 2014). In fact, inoculation with *Azotobacter* has been found to exert several beneficial effects on plant yields as possess various plant growth promoting traits and also found to produce exopolysaccharides under saline conditions (De la Vega et al. 1991; Mrkovacki et al. 1996). Consequently, it has been implicit that isolating bacteria with PGP traits from naturally saline environments would give indigenous isolates to improve the effect of salt stress on plants.

7.3 Plant Growth-Promoting Activities of Salt-Tolerant Bacteria

7.3.1 Nitrogen Fixation Under Salt Stress

Nitrogen is the essential macronutrient required for plant growth. Bacteria inhabiting under saline conditions alter some of their activities and pathways to adapt themselves. One of the sensitive activities is the nitrogenase activity, which is affected by extreme saline conditions. Nitrogen fixation was found to be decreased in saline soils as salt stress adversely affects the nitrogenase enzyme activity (Gao et al. 2014). The extent of effects of salinity on denitrification process is dependent on the type of nitrogen compound present in the soil (El-Shinnawi et al. 1982). These stressed conditions disrupt the nitrogen cycle and lead to the disappearance of nitrate (NO^{3-}) from saline soil through denitrification process, resulting in alteration of enzymatic processes (Azhar et al. 1989). Biological nitrogen fixation (BNF) involves the enzymatic reduction of nitrogen to ammonia (NH_3), which acts as the precursor molecule for the biosynthesis of amino acids and other nitrogen-containing biomolecules. Islam et al. (2010) studied the free-living culturable diazotrophic bacteria of paddy soils under salt stress conditions and found that 32 bacteria were positive for acetylene reduction assay (ARA) and the values ranged from 1.8 to 2844.7 nmol ethylene h^{-1} mg^{-1} protein. The study carried out by Chowdhury et al. (2007) on diazotrophic bacterial isolates showed that the predominance of Gram-negative bacteria from the surface-sterilized roots of *Lasiurus scindicus* were capable of fixing nitrogen. Nitrogen-fixing *Bacillus* strains were also obtained from saline lands of Egypt; these strains reduced acetylene in pure culture at 5% NaCl (Zahran et al. 1992).

7.3.2 Phosphate Solubilization

Phosphorus is one of the key nutrients for plants, but a major portion of it is available in insoluble form. Microorganisms play a vital role in solubilizing phosphorous and in increasing the availability of phosphorous to plants. Phosphate-solubilizing microorganisms belonging to genera *Klebsiella*, *Erwinia*, *Rhizobium*, *Achromobacter*, *Aerobacter*, *Enterobacter*, *Pseudomonas*, *Micrococcus*, and *Bacillus* have been reported earlier. However, strains belonging to *Pseudomonads* and *Bacillus* are deliberated as the most proficient phosphate solubilizers (Villegas and Fortin 2002), whereas fungal species such as *Aspergillus*, *Penicillium*, and *Curvularia* and yeast are widely reported to solubilize various forms of inorganic phosphates (Das et al. 2013).

Several researchers have isolated phosphate-solubilizing microorganisms from various niches of saline soils (Sharan et al. 2008; Park et al. 2010; Srinivasan et al. 2012). A salt-tolerant, nitrogen-fixing, and phosphate-solubilizing species *Swaminathania salitolerans* has been isolated from the rhizosphere, roots, and stems of salt-tolerant

mangrove associated with wild rice (Loganathan and Nair 2004). In another study, phosphate-solubilizing bacteria *Alteromonas* sp. and *Pseudomonas aeruginosa* have been isolated from salt-affected soils. These isolates were found to solubilize phosphate under saline conditions, i.e., up to 2M NaCl concentration (Srinivasan et al. 2012). Rosado et al. (1998) and Nautiyal (1999) observed increased phosphate-solubilizing activity of bacteria in the presence of 10% NaCl, but the solubilizing activity decreased with increase in NaCl concentration.

7.3.3 Siderophore Production

Iron is the fourth abundant and essential growth element for all living organisms and perhaps the most important micronutrient used by bacteria for their metabolism. To confiscate and solubilize ferric iron, many microorganisms utilize low-molecular-weight (<1000 Da) compounds with high iron affinity known as "siderophores." Siderophores are produced by rhizospheric bacteria to enhance the growth and development of plants by increasing the availability of iron. Siderophore-producing microorganisms prevailing in the rhizosphere suggest that plants would all become iron deficient in the absence of iron-chelating siderophores (Kloepper et al. 1980). Nine halophilic archaea were isolated from marine salterns for siderophore production (Dave and Desai 2006). Ramadoss et al. (2013) found that *Bacillus halodenitrificans* and *Halobacillus* sp. isolated from saline habitats exhibited siderophore-producing activity.

7.3.4 Indole Acetic Acid (IAA) Production

Some PGPR strains enhance plants' growth and development by modulating the concentration of known phytohormones. Among plant hormones, auxins and ethylene play an essential role in root system development and crop yield. Indole-3-acetic acid (IAA) is the common natural auxin that extensively affects plant physiology. Diverse microbial groups are capable of producing physiological active auxins, which exert pronounced effects on plant growth and its establishment. In order to produce auxin, bacteria use tryptophan as a precursor molecule and convert it into IAA (Etesami et al. 2009). In plants, saline stress often affects the production of IAA and makes them imbalance. Thus, it is important to study IAA-producing rhizobacteria in saline conditions which could facilitate plant growth under salt stress. It has been reported that pre-sowing seeds with phytohormones alleviated the growth-restricted effect of salt stress (Ramadoss et al. 2013). Zahir et al. (2010) isolated IAA-producing halophilic *Rhizobium phaseoli* strains from the mung bean nodules and evaluated their growth parameters in the presence and absence of tryptophan under salt stress conditions. Growth promotion effects were observed, and this might be due to higher auxin production and mineral uptake in rhizosphere, which reduced the adverse effect of salinity.

7.3.5 Lytic Enzyme Production

Lytic enzyme production is one of the indirect approaches for plant growth promotion. A wide array of organisms have been obtained from harsh environments that produce many active and stable enzymes including proteases (Durham et al. 1987), amylases (Horikoshi 1971), lipases (Watanable et al. 1977), etc. Lytic enzymes produced by biocontrol organisms mediate defense against the pathogens and improve plant growth (Vivekananthan et al. 2004). Enzymes that are stable and active at extreme saline conditions are very much in demand for various industrial processes. Shaheen et al. (2008) reported the protease enzyme production by *Bacillus subtilis* at different concentrations of salt (0–6% NaCl). Sivaprakasam et al. (2011) obtained salt-tolerant alkaline protease from *P. aeroginosa* that was capable of enzymatic degradation.

7.4 Mechanism of Salt Tolerance

Salt stress reduces microbial population in the rhizosphere. Microbes that inhabit hypersaline environments experience intense osmotic pressure and thus use "compatible solute strategy" or the "salt-in strategy" to resist salt stress (Etesami and Beattie 2017). Bacteria accumulate compatible solutes and other amino acids under saline conditions (Brown 1976). Some salt-tolerant bacteria can use salt in strategy mechanism and accumulate electrolytes, e.g., K^+ glutamate. Furthermore, enzymes, ribosomes, and transport proteins of these bacteria require high level of potassium for stability and activity. Organic solutes increase the intracellular osmotic strength and stabilize the cellular macromolecules (Lippert and Galinski 1992).

Specific genetic induction is required to accumulate compatible organic solutes in salt-tolerant bacteria (Plemenitas et al. 2014). Intracellular proline was found to increase rapidly in *Bacillus* in response to osmotic stress by NaCl, and the corresponding genes were detected as proB, proA, and proC encoding γ-glutamyl kinase (γ-GK), γ-glutamyl-phosphate reductase (γ-GPR), and pyrroline-5-carboxylate (P5C) reductase, respectively (Chen et al. 2007). Various genes encoding L-aspartokinase (Ask), L-2,4-diaminobutyric acid transaminase (EctB), L-2,4-diaminobutyric acid acetyltransferase (EctA), and L-ectoine synthase (EctC) have been located and found to be involved in the biosynthesis of ectoine in *Halobacillus dabanensis* (Nada et al. 2011). Four genes, viz., *betI*, *betC*, *betB*, and *beta*, were found to be essential for oxidation of choline or choline-*O*-sulfate to glycine betaine organized in one operon (Sevin and Sauer 2014). Other antiporter genes that have been reported in salt-tolerant bacteria are also essential for maintaining the balance of Na^+ and K^+ ions in the cell in order to attain an osmotic equilibrium. This mechanism is accompanied by certain physiological modifications which are required to protect all the metabolic and regulatory functions at high salinity (Eisenberg and Wachtel 1987). Na^+/H^+ antiporters are membrane proteins involved

in pH and Na$^+$ homeostasis in cells that exchange Na$^+$ for H$^+$ (Inaba et al. 2001). The genes that are proved to be involved in halotolerance in bacteria either through knockout studies or through overexpression studies are given in Table 7.1.

7.4.1 Genetic Variations Based on Nha

Sodium hydrogen antiporters transport Na$^+$ or Li$^+$ in exchange for H$^+$ across the cytoplasmic membrane of cell (Alkoby et al. 2014) and maintain intracellular pH homeostasis, detoxification of cells from Na$^+$ ions, regulation of cell volume, and establishment of an electrochemical potential of Na$^+$ ions (Padan 2014). Various Na$^+$/H$^+$ antiporters such as *nhaA*, *nhaB*, *nhaC*, *nhaD*, *nhaP*, *chaA*, *tetA(L)*, and *napA* have been identified in Gram-positive and Gram-negative bacteria (Padan et al. 2001; Majernik et al. 2001). *NhaA* gene responsible for salt tolerance in

Table 7.1 Genes conferring salt-tolerance response in selected bacteria

Strains	Genes	Product	Source
Pseudomonas aeruginosa	nhaP	Na$^+$/H$^+$ antiporter	Inaba et al. (2001)
Sinorhizobium meliloti	relA bet genes betS gene greA Kup	(p)ppGpp synthetase Glycine Betaine/ proline Betaine transporter Transcription cleavage factor Potassium uptake protein	Wei et al. (2004) Pocard et al. (1997) Nogales et al. (2002)
Rhizobium tropici	ntrY, ndvA and ndvB (synthetic gene) nhaA, nhaB, nhaC	Histidine kinase Na$^+$/H$^+$ antiporter	Miller and Wood (1996)
Azotobacter vinelandii	ggpPS	Glucosyl glycerol biosynthesis	Klahn et al. (2009)
Enterobacter ludwigii	nhaA	Na$^+$/H$^+$ antiporter	Kapoor et al. (2017)
Synechocystis	nhaS1, nhaS2, nhaS3, nhaS4, and nhaS5	Na$^+$/H$^+$ antiporter	http://www.ncbi.nlm.nih.gov/nuccore/NC_016514
Aphanothece halophytica	napA	Na$^+$/H$^+$ antiporter	Inaba et al. (2001)
Bacillus subtilis	proA, proB, proC	γ-Glutamyl kinase	Chen et al. (2007)
Enterobacter cloacae	nhaA	Na$^+$/H$^+$ antiporter	Kapoor (2014)
Bacillus aquimaris	nhaA	Na$^+$/H$^+$ antiporter	Kapoor (2014)

Enterobacter sp. has been reported previously (Kapoor et al. 2017). The primary structure of all the abovementioned genes exhibits very weak or no significant homology. This indicates that different transport systems coupling H^+ and Na^+ circulation have developed during evolution. Several genes encoding Na^+/H^+antiporters from different microorganisms have been shown variability by replacing *nhaA* of *Escherichia coli* e.g., *nhaA* of *Vibrio alginolyticus* (Nakamura et al. 1994), *Vibrio parahaemolyticus* (Kuroda et al. 1994), *Bacillus aquimaris* and *Enterobacter cloacae* (Kapoor 2014), *Enterobacter ludwigii* (Kapoor et al. 2017), as well as *nhaB* of *V. parahaemolyticus* (Nozaki et al. 1996), *nhaD* of *V. parahaemolyticus* (Nozaki et al. 1998), *nhaP* of *Pseudomonas aeruginosa* (Utsugi et al. 1998), *nhaC* of *Bacillus pseudofirmus* OF4 (Ivey et al. 1993), *napA* of *Enterococcus hirae* (Strausak et al. 1993), and *mnh* of *Staphylococcus aureus* (Hiramatsu et al. 1998).

Amino acid residues Asp-133, Asp-163, and Asp-164 (Inoue et al. 1995) and His-225 were proposed to be involved in pH sensitivity in *E. coli* for binding of sodium ions (Gerchman et al. 1993). Furthermore, amino acid residues Gly-14, Gly-166, Phe-267, Leu-302, Gly-303, Cys-335, Ser-342, and Ser-369 located in the cell membrane were identified by Nuomi et al. (1997) and found to be essential for the activity of *nhaA* in *E. coli*. In general, 111 amino acid residues were found to be fully conserved in the *nhaA* gene products from different bacteria (Inoue et al. 1995; Vimont and Berche 2000). Our previous study showed that specific insertions/deletions caused major variations of amino acids in salt-tolerant strains (Kapoor et al. 2017). However, these types of alleles mined the rare mutation among the salt- and non-salt-tolerant strains, and little information is available on allele mining of genes responsible for salt tolerance.

7.5   Conclusions

The salt tolerance mechanism of plants modulated by rhizosphere bacteria opens up new prospects to understand plant–microbe interaction. PGPR strains have been conventionally used as biofertilizers to augment the growth and yield of different crops under salt stress conditions. The variability in salt tolerance behavior of bacteria can be explored by targeting the genes involved in salt tolerance mechanism. Genetic and genomic studies used to determine allele mining in gene sequences among salt- and non-salt-tolerant strains are yet to be explored. Gene silencing approach can be used to study the precise function of specific gene in salt-tolerant strains.

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