# Chapter 55

# Biocontrol and Osmoprotection for Plants under Salinated Conditions

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## 55.1 INTRODUCTION

Today, world agriculture faces an increasing threat by plant pathogens. This can hardly be overcome by conventional methods of pest management. Not only do synthetic pesticides show limited efficiency because of the development of resistance by the pathogen, consumers are also concerned more and more about their effects on environmental sustainability, food safety, and food quality. On the other hand, insufficient food supply and deficiencies of vitamins and micronutrients are widely spread problems in many developing countries and extensive and expensive agricultural efforts are required to address these problems. In many of these areas, soil salinization—originally caused by humidification because of the clearing of trees for agriculture and amplified by salt brought in by ground water and strong irrigation—is an enormous additional problem. In 1999, 42% of arable land in Asia and 31% in the Middle East and North Africa were irrigated; irrigated land in developing countries is estimated to increase by 27% between 1996 and 2030. However, soil salinization is reducing the world's irrigated area by 1–2% every year, affecting most severely the arid and semi-arid regions (FAO, 2005). Climate change may exacerbate this negative tendency. As a result of soil salinization, saline or water imbalance stress renders crops more vulnerable to diseases caused by pathogens, especially by soil-borne fungi.

For many decades, fumigation with methyl bromide and related compounds was the standard method for disease control of soil-borne fungi. However, the complete destruction of microbial communities by methyl bromide leads to a vacuum effect in the soil allowing uncontrolled spread of pathogens unaffected by methyl bromide treatment or introduced into the fields via plant seeds or seedlings (Ibekwe et al., 2001). Furthermore, methyl bromide is a greenhouse gas and the bromine released from methyl bromide depletes ozone in the stratosphere 60 times more efficiently than chlorine (WMO, 1998). It is obvious that the problems mentioned

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above can only be solved by replacement of methyl bromide, which is now forbidden in most countries by environmentally sound solutions, which include the use of consumer-friendly biologicals.

Biological inoculants based on naturally occurring antagonists are an environmentally friendly alternative to control soil-borne pathogens in the rhizosphere (Lugtenberg and Kamilova, 2009; Berg, 2009; see Chapter 54). Under salinated conditions, root-associated beneficial microorganisms can help improve plant growth and nutrition. The exploration of bacterial inocula for alleviation of salt stress and for plant-growth promotion in saline soils has just started, but the first reports are promising (Mo et al., 2006; Egamberdieva et al., 2008; Nadeem et al., 2010). While the mode of action for biocontrol agents is well-investigated (Compant et al., 2005; Lugtenberg and Kamilova, 2009; see Chapter 54), less is known about the osmoprotective function of rhizosphere microorganisms. In this chapter, we describe (i) screening strategies for salt-tolerant BCAs, (ii) examples of their biocontrol and osmoprotective effects, and (iii) the effect and mode of action of *Stenotrophomonas rhizophila*, a model BCA for salinated conditions, in more detail.

## 55.2 SCREENING STRATEGIES, BIOCONTROL AND OSMOPROTECTIVE EFFECTS OF SALT-TOLERANT BCAS

Inconsistent effects under field conditions are one of the main problems in translation of biocontrol studies into practical approaches. To overcome this hurdle, ecological knowledge about BCAs and their interactions with abiotic and biotic factors is necessary (Köberl et al., 2011). This knowledge should be included already in screening strategies.

Different screening strategies were reported and assessed by various research groups. Egamberdieva et al. (2008) isolated salt-tolerant bacteria with potentially beneficial traits from the rhizosphere of Uzbek wheat cultivars. Eight strains were salt-tolerant and promoted growth of wheat. Surprisingly, partial sequencing of the 16S rDNA showed that all eight new isolates were opportunistic human pathogens, e.g., *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Bacillus cereus*, *Enterobacter hormaechei*, *Pantoea agglomerans*, and *Alcaligenes faecalis*. The occurrence of potential human pathogens under extreme conditions is known from other studies too (Berg et al., 2005). This enrichment of opportunistic human pathogens was avoided by Egamberdieva and Kucharova (2009), who isolated salt-tolerant root colonizing rhizobacteria of wheat after the third cycle of enrichment. Here, the five selected strains were identified as non-pathogenic *Pseudomonas putida*, *P. extremorientalis*, *P. chlororaphis*, and *P. aurantiaca*.

Another strategy suggested by Egamberdieva et al. (2011) includes salt tolerance tests in the first step. Interestingly, the majority of rhizobacterial strains were highly tolerant to salt. As the activities of roots and rhizobacteria modify the osmolality of the rhizosphere, osmoprotective mechanisms will influence both the survival of rhizosphere bacteria and their interactions with plant roots (Miller and Wood, 1996). Fifty two beneficial and salt-tolerant bacteria from different strain collections from all over the world were screened for their ability to promote growth and/or to control diseases caused by the soil-borne fungus *Fusarium solani* on cucumber and tomato plants. The five most effective strains were used in large-scale greenhouse trials. Four out of five strains significantly controlled cucumber foot and root rot, reducing the percentage of diseased plants from 54% in the untreated control to between 10% and 29% in bacterized plants. All five strains increased the dry weight between 29% and 62%. In two consecutive years, all five strains significantly increased the plant height (by 4–15%) as well as the fruit yield (by 12–32%). The conclusion was that many beneficial bacteria isolated from plants grown on non-salinated soil are perfectly able to promote plant growth and control plant diseases in salinated soil. *Stenotrophomonas rhizophila* strain DSM14405T, which was originally isolated from the rhizosphere of oilseed rape in Northern Germany, was one of the most effective strains in this study.

ACC (1-aminocyclopropane-1-carboxylic acid)-deaminase activity is another interesting target for screening strategies of BCAs active under salt stress. This was used by Nadeem et al. (2010) to evaluate rhizobacterial strains by conducting a jar experiment under axenic conditions at different salinity levels. In general, salinity reduced the growth of wheat, but inoculation with selected rhizobacteria improved the growth and yield compared with the non-inoculated controls. At a high salinity level (15 dS $m^{-1}$), the inoculants increased plant height, root length, plant biomass, and grain yield by 37%, 70%, 116%, and 111%, respectively. Inoculated plants had higher $K^+/Na^+$ ratios and higher relative water and chlorophyll contents. However, proline contents of inoculated plants were lowered compared to the controls. *Pseudomonas putida* W2 and *P. fluorescens* W17 alleviated salt stress most effectively, even at higher salinity levels. The results suggest that the assay for ACC-deaminase activity could be a first step in screening for effective plant-growth-promoting rhizobacteria (PGPR) capable of increasing the growth and yield of wheat under salt stress.

For biocontrol approaches in salinated soils, salt tolerance of BCAs is an important requirement (Príncipe

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et al., 2007; Egamberdieva et al., 2011). Apart from antagonistic traits, production of osmoprotective substances or ACC deaminase is essential for successful introduction into salinated soils. However, the study of Egamberdieva et al. (2011) showed that salinization does not seem to be a threat for the application of currently used plant-beneficial bacteria because many BCAs are salt-tolerant generalists and can be applied under salinated conditions. This was also shown for the two main BCAs toward soil-borne pathogens: *Pseudomonas* (Rangarajan et al., 2003; Diby et al., 2005) and *Bacillus* (Bochow et al., 2001). There are hints that especially salinated soils contain potential human-pathogenic pathogens; this is an additional reason to integrate biosafety aspects at an early stage of product development. Here, biological assays for human pathogenicity, such as growth at 37 °C and the *Caenorhabditis elegans* assay (Zachow et al., 2009), should be used for risk assessment.

## 55.3 *Stenotrophomonas rhizophila*: A MODEL BACTERIUM FOR SALT-TOLERANT BCA

*Stenotrophomonas* isolates have a great potential for applications in biotechnology and biological control because of their high capacity to promote plant growth and their antagonism against various plant pathogenic fungi (Ryan et al., 2009; Berg et al., 2010). The species *S. maltophilia* has become important as a nosocomial multidrug-resistant human pathogen associated with significant case/fatality ratios, particularly in patients who are severely debilitated or immunosuppressed. On the contrary, no pathogenic potential for humans has ever been observed in the closely related species *S. rhizophila* (Wolf et al., 2004). Both species can be easily distinguished by the production of osmoprotective substances (only present in *S. rhizophilia*) and the occurrence of multidrug-efflux pumps (only present in *S. maltophilia*) (Ribbeck-Busch et al., 2005).

Plant-growth promotion by *S. rhizophila* strain DSM14405$^T$ (syn. strain e-p10) was also observed in the highly-salinated soils of Uzbekistan. In comparison to a non-treated control, *S. rhizophila* seed treatment significantly increased the germination rate and shoot and root growth of wheat, tomato, lettuce, sweet pepper, melon, celery, and carrot. In tomato, the germination rate was 180%, the growth of the shoot 120%, and that of the root 142% in comparison to the non-treated control (Egamberdieva, *personal communication*; Egamberdieva et al., 2011). Plant species-specific effects were also observed for *S. rhizophila* DSM 14405$^T$ at increasing salinities under greenhouse conditions. Plant-growth promotion was particularly apparent in solanaceous crops (tomato and sweet pepper) in contrast to cotton. Under greenhouse conditions, a positive effect of *S. rhizophila* DSM14405$^T$ on the growth of sweet pepper was consistent across all tested salinity values. In general, plant-growth promotion by *S. rhizophila* DSM14405$^T$ was more pronounced in non-sterile (greenhouse and field conditions) than in sterile soil, which can be explained by the interaction with indigenous plant-associated microorganisms.

Use of classical physiological and biochemical methods unveiled the mechanisms of plant-growth promotion and biocontrol by *Stenotrophomonas* against soil-borne fungi such as *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Verticillium dahliae*. Biocontrol involves the excretion of antifungal metabolites such as antibiotics, toxins, and biosurfactants, and the production of a wide range of extracellular enzymes (Jacobi et al., 1996; Berg and Ballin, 1994; Kobayashi et al., 1995; Dunne et al., 2000). The excretion of soluble antibiotics and enzymes as well as the production of volatile organic compounds (VOCs) by soil bacteria such as *Stenotrophomonas* can negatively influence the growth of fungi (Alström, 2001; Wheatley, 2002). Recently, it has been shown that the VOCs of *S. maltophilia* and *S. rhizophila* inhibit mycelial growth of the soil-borne pathogen *R. solani* by more than 90% in dual culture tests. Out of a vast diversity of VOCs produced by *S. rhizophila*, two, namely dodecanal and α-phenylethanol could be identified by GC-MS (Kai et al., 2007). It has been shown that *S. rhizophila* has a high-potential for plant-growth promotion although positive effects are dose-dependent (Wolf et al., 2004; Suckstorff and Berg 2003). *S. rhizophila* is able to grow at salt concentrations up to 4% and produces the osmolytes trehalose and glucosylglycerol (GG) in response to salt stress (Roder et al., 2005). Osmolytes are compounds compatible with cellular functions, e.g., DNA replication, DNA-protein interactions, and cellular metabolism. Osmolytes are highly soluble; they have no net charge at physiological pH and do not interact with proteins. Furthermore, they function on osmotic balance and are effective stabilizers of enzymes (Lippert and Galinski, 1992; Welsh, 2000).

Genome sequencing resulted in new insights into the genetic sources providing beneficial plant-associated bacteria with traits such as plant-growth promotion, antagonisms toward phytopathogens as well as osmoprotection. Here we discuss some of these genetic sources for *S. rhizophila* DSM14405$^T$. *S. rhizophila* produces high levels of the auxin phytohormone indole-3-acetic acid (IAA) (Suckstorff and Berg, 2003). Bacteria synthesize IAA through various pathways (Spaepen et al., 2007). Our BLASTn investigations revealed that the genes encoding the regular pathways of IAA synthesis such as tryptophan-2-monooxygenase and tryptophan

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decarboxylase are not present in the genome of *S. rhizophila*. However, the presence of the nitrilase gene suggests that the biosynthesis of IAA in *S. rhizophila* could be through the indole-3-acetonitrile pathway (see Chapter 29). Moreover, *S. rhizophila* possesses genes responsible for the synthesis and transport of osmoprotective molecules (osmolytes) out of the cell. The glucosylglycerol-phosphate synthase (*ggpS*) gene has been shown to be essential for the synthesis of the osmolyte GG, which has been suggested to be transported into the environment by a transporter encoded by the *ycaD* gene located upstream of *ggpS* (Hagemann et al., 2008). *S. rhizophila* also possesses a number of genes known to be involved in the biocontrol of soil-borne pathogens such as the one encoding a metalloprotease and some antibiotic synthesis genes. Furthermore, our genome sequence investigations have revealed that *S. rhizophila* possesses a novel gene homologous to the one coding for the bacterial lanthionine synthetase C-like protein (LanC). The bacterial *lanC* is responsible for the synthesis of peptide antibiotics (lantibiotics). Subtilin from *Bacillus subtilis* ATCC 6633 and epidermin from *Staphylococcus epidermidis* are two other well-studied examples for bacterial metabolites with antibiotic activity against other bacteria (Chung et al., 1992; Schnell et al., 1992). Aside from well-known products such as IAA and VOCs there also exist rather backbone mechanisms leading to plant-growth promotion and biocontrol (Egamberdieva et al., 2011). The bacterial competitiveness for colonizing niches and utilizing nutrients are important examples of these mechanisms (Kamilova et al., 2005). *S. rhizophila* possesses several genes of great importance with regard to competition for nutrients and niches, which are not present in other plant-associated *Stenotrophomonas* spp. such as *S. maltophilia* R551-3. Examples are genes responsible for pectin degradation, xylan degradation, and the pectate lyase gene. Adhesion to the host–plant cell surface and the ability to form biofilms are also important features for out-competing other microorganisms during the competition for niches (see Chapter 66). To this end, *S. rhizophila* is equipped with several genes which may play a role in root colonization, such as those encoding the O-antigen and capsule polysaccharide biosynthesis pathways, genes encoding hemagglutinin, outer membrane adhesion protein, etc. Furthermore, there are also amino acid-biosynthesis genes present in the genome of *S. rhizophila* such as the one encoding chorismate mutase, which plays a crucial role in the biosynthesis pathway of tyrosine and phenylalanine (Guo et al., 2001). In addition, our BLASTp analyses have revealed that *S. rhizophila* possesses a gene homologous to the bacterial cardiolipin synthetase (*cls*), which plays an important role in the adaptation to elevated osmotic stress because of environmental changes (Romantsov et al., 2008). Figure 55.1 shows an overview of the features of *S. rhizophila* DSM14405T, which may play a role in plant-growth promotion, biocontrol and plant osmoprotection.

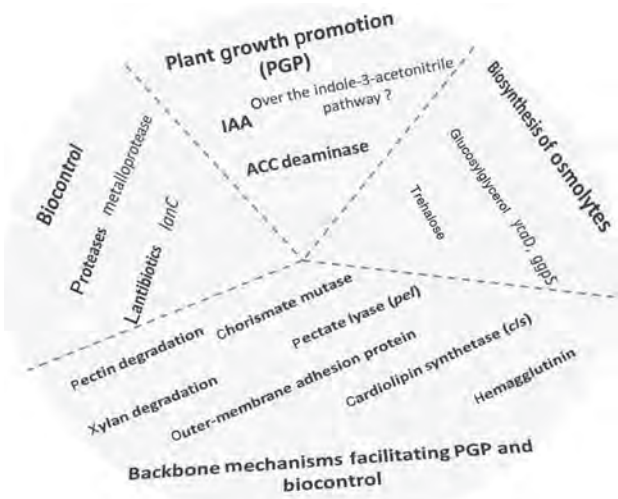



**Figure 55.1** Overview of features of *S. rhizophila* DSM14405T, which may play a role in plant-growth promotion, biocontrol, and plant osmoprotection. Features and corresponding genes were detected through genome sequence investigations, BLASTp analysis, as well as through references cited in this work.

## 55.4 CONCLUSIONS

Root-associated bacteria can protect plants against abiotic and biotic stress under salinated conditions. Regarding plant-growth promotion, antagonistic traits and high salt tolerance, production of osmoprotective substances as well as ACC deaminase activity are key factors involved in the mode of action. Therefore, these traits should be included in screening strategies. However, further research is necessary to understand the plant–microbe interaction under salinated conditions and to optimize biocontrol and stress protection products.

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