

Chapter 21

Halophyte–Endophyte Interactions: Linking Microbiome Community Distribution and Functionality to Salinity



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Abstract Many plants are unable to adapt to rapid environmental changes (e.g., salinity, drought, or limited nutrients) and may acquire assistance from microbes that have the capacity to increase tolerance of host-plants in stress conditions. By having the right microbes, the plants are more resilient! Such microbes include endophytes that inhabit inner tissues of the plant without causing symptoms of disease in their host. However, this plant–endophytic association exists only when chemical equilibrium is maintained between both, therefore making this mutualistic interaction even more unique. Therefore it is interesting to decode the endophytic community composition in halophytes specifically in the most salt-tolerant halophyte species *Salicornia europaea*, and further determine the factors that could affect this association. Moreover, understanding the endophytes potential plant growth-promoting activities in association with host (*S. europaea*) and non-host plant (non-halophytes) are the focus of this chapter.

Keywords *Salicornia europaea* · Non-mycorrhizal plant · Salt tolerance · Bacteria · Fungi · Proteobacteria · Ascomycota · Soil salinity · Culture dependent · Metagenomic · Enzyme activity

21.1 Introduction

Soil salinity is among the major abiotic stresses affecting crop production today. It is caused due to climate change, low precipitation, high surface evaporation, weathering of native rocks, improper irrigation management on landscapes, salts used for deicing roads, and poor agricultural practices (Litalien and Zeeb 2019). Soil salinization is characterized by a high concentration of soluble salts, when the

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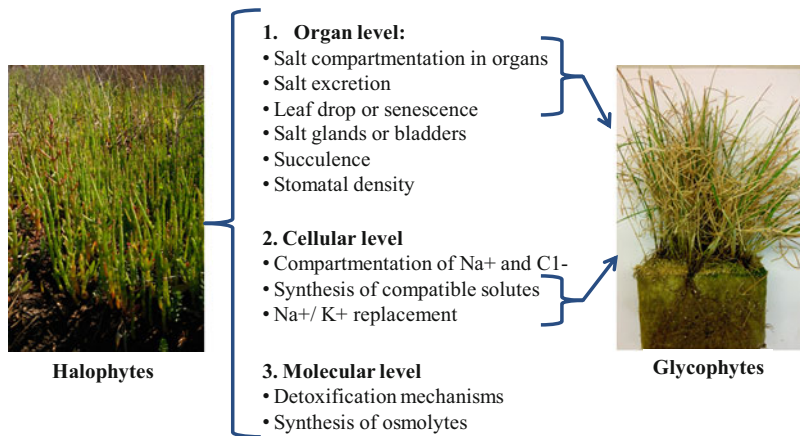


Fig. 21.1 Response of halophytic and glycophytic plants under salinity stress at the (1) organ, (2) cellular, and (3) molecular levels. Halophytes possess all of the mechanisms to combat salinity stress while glycophytes have minimal tolerance to stress *via* organ and cellular responses only and no mechanisms at the molecular level

electrical conductivity (EC_e) in the root zone exceeds 4 dS m^{-1} (approximately 40 mM NaCl) at 25°C and has exchangeable sodium of 15% and osmotic pressure of approximately 0.2 MPa (reviewed by Munns et al. 2020). The yield of most crop plants is reduced at this EC_e , though many crops exhibit yield reduction at lower EC_e (reviewed by Munns et al. 2020). Salt stress damages various physiological and metabolic processes in plants by inducing osmotic stress and increasing ion toxicity, nutrient deficiency, membrane disruption, and inhibition of metabolic activities, as well as changes in gene expression (Shahzad et al. 2019).

Nevertheless, there are some plants that show no evidence of inhibition in extreme salinity, and normally exhibit a pronounced salt requirement for optimal plant growth (Nikalje et al. 2019). These plants are called halophytes, which are representative vegetation of saline habitats ranging from coastal regions, salt marshes and mudflats, saline depressions, inland deserts and sand dunes and rocky coasts (Nikalje et al. 2019). Most of the halophytes belong to family Amaranthaceae, Plumbaginaceae, Plantaginaceae, Aizoaceae, Poaceae, Brassicaceae, with the Chenopodiaceae being dominant (Slama et al. 2015). The database “eHALOPH” currently identified more than 1500 halophyte species reported from different parts of the world. Clearly, halophytes have evolved a number of adaptive traits expressed at various levels of organization (Fig. 21.1) that includes adjustment of their internal water relations through salt exclusion, succulence, salt-secreting glands and bladders, ion compartmentation in cell vacuoles and accumulation of compatible organic solutes and are distinguishable from glycophytes (salt-sensitive plants) (reviewed by Fan 2020).

It is inevitable that no organism thrives alone, which questions the probable existence and role of the closely associated microbiome present in halophytes and

their contribution toward the halophytes' high salt tolerance ability. Endophytic microorganisms ubiquitously occur in all plant species and are important components of the biological diversity. The term “endophyte” can be defined as all organisms that at some point in their life cycle colonize internal plant tissues without causing harm or symptoms of disease in their plant host (Petrini 1991). These include bacteria, actinomycetes, or fungi that colonize the internal plant tissues. Endophytes are particularly fascinating because of their multifaceted lifestyle, i.e., they may exist as either free-living soil microbes or saprobes or pathogens (Tadych et al. 2009). They exhibit variations in their mechanisms of transmission from one plant host to another including strict vertical transmission from maternal plants to seeds, infectious transmission from one host plant to another, or infection by spores from environmental sources like wind, rain, soil, and leaf litter (Tadych et al. 2009). Endophytes are known to share a symbiotic relationship with their plant host, wherein they use the internal environment of the plant as an ecological niche, and in return protect their host from the negative effects of the adversely changing environment (Petrini 1991). Alternatively, some endophytic species could be neutral and do not offer any benefits or neither harm their hosts (Backman and Sikora 2008). With this background, this chapter discusses on the diversity of endophytic bacterial and fungal communities in halophyte *Salicornia europaea* found in different salinity environments. It is a well-known fact, that the true endophyte state in the host plant is established only when the chemical equilibrium between the host and microbe is achieved during their long-term association. During this process of endophyte stabilization in host plant, many environmental factors such as abiotic (e.g., temperature, salinity, soil composition) and biotic (e.g., competition with microbes already present in the host) could alter or redefine the metabolic capacity of endophytes. Hence, we assess on the possibility of these endophytes possessing beneficial traits or properties related to plant growth promotion or stress tolerance in their host further on their resilience when inoculated in other non-host plants. Further, we underlie the importance of understanding the endophytic microbiome in halophytes which will likely pave a new avenue in engineering endophyte-mediated stress tolerance in plants.

21.2 *Salicornia* a Potential Halophytic Crop Plant

The halophyte chosen for this discussion, i.e., *Salicornia europaea* L. (Amaranthaceae) is a non-mycorrhizal plant (Sonjak et al. 2009) and one of the most salt-accumulating halophyte known. It is a “pioneering plant” in both coastal and inland saline sites, which has generated significant interest as a multi-purpose halophyte (Nikalje et al. 2018). *S. europaea* grows up to a height of 35 cm and is fairly richly branched. They are dark green becoming yellow-green and ultimately flushed pink or red (Ventura and Sagi 2013). They are succulent annuals with extremely reduced leaves and a spike-like terminal inflorescence (Ventura and

Sagi 2013). This genus is widely dispersed in Eurasia, North America, and South Africa, presently comprises around 25–30 species (Ventura and Sagi 2013).

S. europaea is a crop with great commercial value and ecological importance. It is suitable for cultivation as a vegetable in highly saline environments (Ventura and Sagi 2013) and as a source of valuable secondary compounds (Singh et al. 2014). *Salicornia* spp. has been successfully grown in aquaculture systems (Singh et al. 2014). As a salt-accumulating halophyte, as much as 50% of the dry weight of *S. europaea* may be salt ions (Ushakova et al. 2005; Furtado et al. 2019a). This was also observed in the results of Furtado et al. (2019a) where high accumulation of Na⁺ in shoots, whereas K⁺ and Ca²⁺ levels were higher in roots throughout the sampling period (spring and autumn) in all *S. europaea* samples collected from the two salt-affected sites. Therefore, this species is promising for soil desalination, which is required for the development of agriculture on salty soils and beaches. Moreover, research on halophyte plants is of particular interest today not only for their high salt tolerance and agronomic value but also because it is a non-mycorrhizal plant. This fact makes it interesting to discuss their associated microorganisms (endophytes) that could compensate for the missing symbiotic protection and increase plant growth and fitness particularly under unfavorable conditions. *S. europaea* is especially known to produce secondary compounds (e.g., alkaloids, triterpenoid saponins, and flavonoids, among others) (Isca et al. 2014). Many of these compounds are used as energy sources by endophytic bacteria and fungi suggesting that fluctuations in the proportions and type of compounds during the plant development play an active role in selection for specific bacteria and/or fungi.

21.3 Biodiversity of Endophytic Microbiome in *S. europaea*

The endophytic community in halophytes may be different from those in other plants because salinity acts as an environmental filter. Thus, allowing the survival of selected taxa that can withstand extreme environments. To date, endophyte community diversity investigated in *S. europaea* have been analyzed *via* culture-independent approaches, e.g., sequencing of the 16S rRNA gene and/or the internal transcribed spacer regions (ITS1 and ITS2), 454 pyrosequencing (e.g., Shi et al. 2015; Szymańska et al. 2016; Zhao et al. 2016a; Szymańska et al. 2018; Yamamoto et al. 2018; Hryniewicz et al. 2019; Furtado et al. 2019a) or culture-dependent approaches involving procedures for surface sterilization of plant tissue followed by fragmentation and culture of the fragments onto specific agar plates (e.g., Potato Dextrose Agar, R2A media, Czapek dox agar, etc.) amended with antibiotics (e.g., tetracycline, Nystatin, etc.) (e.g., You et al. 2014; Okane and Nakagiri 2015; Park et al. 2016; Zhao et al. 2016b; Furtado et al. 2019b).

21.3.1 *Salicornia* Bacterial Diversity

Prokaryotic endophytes are considered to be diverse comprising of phyla bacteria and a small portion of Archaea. Overall, the bacterial endophytes are found across many phyla, generally the most abundant community is Proteobacteria (group of Gram-negative bacteria), including α , β , and γ -Proteobacteria classes followed by Firmicutes, Actinobacteria, and Bacteroidetes. Using the culture-independent approach, the bacterial community in the rhizosphere soil and endophytes of *S. europaea* growing at the Fukang Desert Ecosystem Observation and Experimental Station (FDEOES) in Xinjiang Province, China were investigated (Shi et al. 2015). Proteobacteria was most dominant phylum in all the samples followed by other large phyla Firmicutes, Bacteroidetes, and Actinobacteria. This study is consistent with results obtained in Fukang, China (Zhao et al. 2016a), Ciechocinek and Inowroclaw, Poland (Szymańska et al. 2018), Lake Notoro in the eastern part of Hokkaido, Japan (Yamamoto et al. 2018), Ciechocinek and Inowroclaw, Poland (Furtado et al. 2019a). Szymańska et al. (2018) suggested that the endophytes representing phyla Proteobacteria and Bacteroidetes predominate in saline environments regardless of the level of salinity in the root zone soil and plant roots. It was also found that bacteria representing Actinobacteria exhibited lower tolerance to salt stress and this phylum exists more frequently in environments with lower levels of salinity (e.g., Shi et al. 2015; Szymańska et al. 2018; Yamamoto et al. 2018).

This is contrary to the results obtained *via* culture-dependent methods in which phylum Firmicutes and Actinobacteria dominate in many reports. Zhao et al. (2016b) isolated endophytic bacteria of *S. europaea* growing at Gurbantünggüt Desert, China. These isolates belonged to phylum Firmicutes and Actinobacteria with 13 different bacterial genera *Arthrobacter*, *Bacillus*, *Brachy bacterium*, *Brevibacterium*, *Glycomyces*, *Isoptericola*, *Kocuria*, *Mesorhizobium*, *Pseudomonas*, *Phyllobacterium*, *Planococcus*, *Streptomyces*, and *Variovorax*. Another showed Gram-positive bacteria (phylum Firmicutes and Actinobacteria) dominating in all experimental variants including genera *Bacillus* sp., *Streptomyces* sp., and *Microbacterium* sp. (Szymańska et al. 2016).

Some microbes are ubiquitous and can exist as free-living soil microbes, as epiphytes or as endophytes. For instance, *Bacillus* sp., *Salinicola* sp., *Serratia* sp., *Streptomyces* sp., *Microbacterium* sp. and *Rhodococcus* sp. were reported as endophytes in *S. europaea* shoot and roots collected from two sites in Poland (Furtado et al. 2019b) and similar bacterial diversity was previously obtained in the rhizosphere samples at the same investigated sites (Szymańska et al. 2016). Although another study showed no significant differences in bacterial diversity and richness between the bulk soil, rhizosphere, and the root endosphere for *S. europaea* but the differences were observed at the genera level with the most abundant root endophytes including *Sulfurimonas*, *Coleofasciculus*, and *Aestuariusispira* while, *Roseovarius*, and *Halochromatium* were highly abundant in the rhizosphere and seven genera were dominant the *S. europaea* bulk soil: *Thiogrimum*, SEEP-SRB1, *Caldithrix*, *Ignavibacterium*, Sva008 sediment group, Candidatus Thiobios, and

Spirochaeta (Yamamoto et al. 2018). Overall, the bacterial endophytic community in *S. europaea* is much greater in comparison to the fungal community according to results obtained by Szymańska et al. (2016) and Furtado et al. (2019b).

21.3.2 *Salicornia* Fungal Diversity

Although the endophytic microbiome may comprise a small portion of fungi (compared to bacteria) their contribution can be essential in the plants development. Notably, class 2 and class 4 fungal endophytes are commonly found in halophytes, are capable of extensive tissue colonization and are relevant for plant survival in stress habitats (Flowers and Colmer 2015). Moreover, the class 2 fungal endophytes can establish habitat-adapted symbiosis and confer specific stress tolerance to their host and non-host plants in extreme habitats (Flowers and Colmer 2015).

Furtado et al. (2019a) performed metagenomic analysis of *S. europaea* shoots and roots obtained from two different high-salinity environments in Poland and showed that 95% of the fungal reads belonged to phyla Ascomycota. In the next study, the culturable endophytic fungal diversity was investigated by Furtado et al. (2019a) which isolated 320 fungal strains mainly represented phylum Ascomycota (96% of the isolates) from the roots and shoots of *S. europaea* in the same sites. The endophytic fungal strains mainly consisted of the orders: Pleosporales (dominated by *Alternaria* sp. and *Stemphylium* sp.), Eurotiales (mainly *Aspergillus* sp. and *Penicillium* sp.) and Hypocreales (only *Fusarium* sp. and *Trichoderma* sp.). The remaining genera represented the order Dothideales, *Unclassified*, Capnodiales, Sordariales, Botryosphaerales, and Chaetothyriales. Okane and Nakagiri (2015) found *Pleospora* sp. and *Alternaria alternata* were the major endophytes of *S. europaea* roots in the eastern Hokkaido and the Seto Inland Sea (Setouchi) regions in Japan. Endophytic fungal isolates belonging to 9 genera: *Aspergillus*, *Penicillium*, and *Fusarium* were dominantly distributed genera in roots of *S. europaea* native to saltern of the Korea, followed by *Aureobasidium*, *Cladosporium*, *Gibberella*, *Macrophoma*, *Phoma*, *Stemphylium*, and unidentified (Pleosporales), respectively (Park et al. 2016). Alike, Booth et al. (1988) reported a high frequency of *Alternaria* species isolated from *S. europaea* in southern Manitoba and Saskatchewan, Canada. Furthermore, the dominance of *Alternaria* sp. found in *S. europaea* was also previously reported in Canada (Muhsin and Booth 1987), South Korea (You et al. 2014), Japan (Okane and Nakagiri 2015) and Poland (Furtado et al. 2019b). To date, most of the fungal strains (e.g., *Epicoccum* sp., *Alternaria* sp., *Phoma* sp., *Fusarium* sp., *Cladosporium* sp., *Penicillium* sp., *Acremonium* sp., *Lewia* sp., Pleosporales sp., *Stemphylium* sp. and *Aspergillus*, etc.) reported in *S. europaea* belong to a group of common plant pathogens and saprobes found in other plants (Okane and Nakagiri 2015; Park et al. 2016; Furtado et al. 2019b). However, colonization of these endophytes in healthy halophyte plant tissue indicates they are not pathogenic and we propose that these fungi may have “co-evolved” in their host. Few studies have

discussed the abilities of fungi to switch lifestyles between the endophyte-pathogen (Hyde and Soyong 2008) and endophyte-saprotroph (Promputtha et al. 2010).

In general, very few endophytic fungal strains belonging to the Phylum Basidiomycota have been reported in *S. europaea*, e.g., a study showed 4% of the isolated diversity were categorized in Basidiomycota into four orders; Polyporales (most representative genus being *Peniophora*) and the other strains corresponded to Russulales, Agaricales, and Cantharellales (Furtado et al. 2019b). These findings were parallel to studies using culture-independent techniques (e.g., Furtado et al. 2019a).

21.4 Factors Shaping *S. europaea*–Endophyte Association

The halophyte–endophyte association is dependent on many factors, mainly the host plant lifestyle (i.e., obligate halophyte *S. europaea* in this discussion) and soil characteristics (e.g., salinity) can have a strong effect on the rate of endophyte colonization (Szymańska et al. 2014; Shi et al. 2015; Zhao et al. 2016a; Szymańska et al. 2018; Furtado et al. 2019a, b). Most endophytic communities are strongly influenced by the soil micro-ecological environment as endophytes are recruited from the soil which may act as a filter for microbial species (e.g., Szymańska et al. 2014; Shi et al. 2015). Research has shown that plants growing in soils with high salt content harbor endophytic assemblages that differ significantly from those in other environments. Two saline sites characterized by different salinity and ion composition (anthropogenic salinity site: lower EC_e with Ca²⁺ and natural salinity site: higher EC_e with Na⁺) showed the greatest abundance of bacteria in the rhizosphere samples than that observed in plant roots (Szymańska et al. 2016). They found majority of rhizosphere bacteria belonged to Firmicutes however; this proportion was lower compared to the endophytes (consisted of 50% Firmicutes). This is in line with previous observations by Shi et al. (2015) where a higher total bacterial biomass was measured in soil, followed by roots of *S. europaea* and the bacterial diversity in the endosphere of *S. europaea* was lower than that in the rhizosphere of *S. europaea* (Shi et al. 2015).

In a second survey, Szymańska et al. (2018) analyzed the endophytic bacterial community in *S. europaea* roots from the same test sites using metagenomic approaches. The results revealed that the higher levels of soil salinity did not reduce the composition of endophytic bacterial diversity in roots. However, a distinct taxonomic composition was observed at the two sites which were attributed to the distinct adaptation of halotolerant microorganisms. This was also found in the culturable diversity study where the highest abundance of bacterial endophytes was isolated from the natural saline site compared to the anthropogenic site and frequency of isolation in the culture medium increased with increasing NaCl concentrations (Szymańska et al. 2016). Thus, indicating the presence of a significant number of halophilic bacteria known to survive at high salt concentrations. This difference indicates that the local environment has a complex effect on the bacterial

community. Supporting this view, a clear influence of soil properties on the existence of unique bacterial and fungal endophytes of *S. europaea*, specific to the geographical regions in different countries, such as Japan (Okane and Nakagiri 2015), Canada (Muhsin and Booth 1987), China (Zhao et al. 2016a, b), South Korea (You et al. 2014) and Poland (Furtado et al. 2019a, b) was demonstrated.

The importance of acclimatization time in adaptation of microorganisms to salinity was emphasized by Szymańska et al. (2016, 2018) and Furtado et al. (2019a, b) when they found that the site exposed to salinity for a long time had a greater diversity of endophytic bacteria and fungi in native *S. europaea*. They propose that time necessary for shaping the bacterial community at the saline sites was longer in the naturally saline area which existed much longer as compared to the anthropogenic salinity area. A study by Furtado et al. (2019a) investigated the composition of endophytic community in *S. europaea* and observed some bacteria were specific in some samples. Bacterial class Sphingobacteriia were found only in the high-salinity site, while Epsilonproteobacteria were characteristic for the site with lower salinity. Some endophytic genera: *Halomonas*, *Levinella*, *Vibrio*, *Pseudoalteromonas*, and *Leuweenhoekiella* were found exclusively at high-salinity site.

Yamamoto et al. (2018) studied the bacterial diversity and community structure of rhizosphere, root endosphere, and bulk control soil samples in two halophytic plants: *Glaux maritima* and *Salicornia europaea*. Among the *G. maritima* samples, the richness and diversity of bacteria in the rhizosphere were higher than in the root endosphere but were lower in comparison to the bulk soil. In contrast to *S. europaea*, the bulk soil, the rhizosphere, and the root endosphere had similar bacterial richness and diversity (Yamamoto et al. 2018). Another study by Shi et al. (2015) showed the diversity of bacteria was abundant in the rhizosphere soil, while the endophytic diversity was poor in *S. europaea* samples. In conclusion, the variation in endophyte frequencies could be due to differences in host preference rather than environmental factors as the halophyte hosts in the abovementioned studies were at the same location and were investigated simultaneously. This also confirms high endophyte species specificity of *S. europaea*, which depends on the halophytic plant species and the sampling site.

Previous data revealed that bacterial communities in the rhizosphere exhibit greater richness than endophytes in the organs of halophytes. In addition, Momonoki and Kamimura (1994) reported that during the growth period of wild *S. europaea* found around Lake Notoro, the pH and osmotic pressure of the plants increased from 7.6 to 8.8, and from 650 to 2000–2600 mOsm/kg (1 mOsm/kg = 17.02 mm Hg), respectively. This fact is clear that the internal environments of halophytes such as *S. europaea* are likely to be stressful to microbes. Much of the endophytic diversity research is limited to the roots of *S. europaea*. However, some studies have indicated plant organ specificity among endophytes in *S. europaea*, where different bacterial and fungal species inhabited tissues and a few species were overlapping between shoots and roots (Furtado et al. 2019a). At the family level, Halomonadaceae were much more abundant in shoots, whereas Alteromonadaceae, Cellvibrionaceae, Flammeovirgaceae, Rhodobacteraceae, and Saccharospirillaceae were characteristic

for roots. *Kushneria* sp. was abundant genus in shoots while *Saccharospirillum* was significantly more common in the roots. In case of fungi, roots at the high-salinity soils were strongly dominated by Pleosporaceae. Family Leptosphaeriaceae, Teratosphaeriaceae, and Didymosphaeriaceae were found exclusively in shoots, and *Paradendryphiella arenariae* was the only species present in all sample types (Furtado et al. 2019a). Moreover, *Paradendryphiella arenariae* (Nicot) Woudenberg and Crous, was previously found in *S. europaea* in Hokkaido (Okane and Nakagiri 2015) and in Canada (as *Dendryphiella arenariae* Nicot) (Booth et al. 1988). In general, the endophytic community (both bacteria and fungi) in *S. europaea* shoots was found to be less diverse than in roots (*S. europaea*). One of the reasons being that the endophyte colonization is dependent on the plant host, that is, imposed by a large salt concentration in this organ (*Salicornia* shoot accumulate salts). Secondly, these differences in endophytic assemblages in different tissue types might be the preferences of individual dominating taxa and may reflect on their capacity for utilizing or surviving within a specific substrate.

Zhao et al. (2016a) observed a marked difference in endophytic bacterial communities from different stages of plant growth. The richest endophytic bacteria diversity of *S. europaea* was detected at the seedling stage, and thereafter, variety of endophytic bacteria declined during flower and fruit setting stage. Phylum Gammaproteobacteria increased during the growing period while Betaproteobacteria decreased. Five genera *Serpens*, *Halomonas*, *Pseudomonas*, *Azomonas*, and *Pantoea* were observed during all growth phases which were suggested as the core-microbiome of *S. europaea*. The fungal diversity in *S. europaea* reported by Furtado et al. (2019b) changed from one growing season to another. The fungal strain from two investigated sites was more diverse (based on genera) in spring (young plants) than in autumn (mature plants) sampling. Certain genera (e.g., *Aureobasidium*, *Cladosporium*, *Epicoccum*, and *Talaromyces*) occurred only in the autumn, while *Neocamarosporium*, *Ascochyta*, and *Acremonium* in spring. The ease of colonization in the young host plant stage and the microorganism's potency to compete for survival in their host may account for this effect.

21.5 *S. europaea* as a Reservoir of Specialized Endophytic Diversity

Soil salinity not only affects microbial community composition and abundance but also affects microbial functions, i.e., enzymatic and metabolic processes. High salinity can reduce the level of respiration, biomass, and activity of microorganisms (Szymańska et al. 2014). For example, the activity of endophytes was found to be higher in sites with lower salinity than at the site with high salinity. Further the endophytes isolated from different samples (different sites and plant organ) although in the same genera *Epicoccum* sp., *Arthrinium* sp., and *Trichoderma* sp. displayed

different metabolic activities (Furtado et al. 2019b). Moreover, different microbial species have specific salt requirements and possess varying tolerance levels. Based on their ability to grow in the saline environments, microbes can be grouped as halotolerant (tolerate up to 25% NaCl) and halophilic (require salt for growth) (Sultanpuram and Mothe 2019). Bacterial and fungal endophytes of *S. europaea* are classified halotolerant as experimentally tested by Szymańska et al. (2016, 2018) and Furtado et al. (2019b). The halotolerant microbiome can positively affect the halophyte by providing nutrients (e.g., atmospheric nitrogen fixation, phosphorus solubilization), producing hormones (e.g., 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and indole-3-acetic acid (IAA)), regulating antioxidant response, synthesizing exopolysaccharides (EPS), maintaining plant defense against biotic stress (e.g., production of antibiotics, competition with pathogens for nutrients, and induction of systemic resistance), accumulating organic solutes such as proline and betaine and increasing soil aggregation (Zhao et al. 2016b; Szymańska et al. 2014, 2016, 2018; Piernik et al. 2017; Hryniewicz et al. 2019; Furtado et al. 2019b).

Secretion of phytohormones, particularly indole-3-acetic acid (IAA), increases plant salt tolerance by stimulating root proliferation. IAA-secreting endophytes have been isolated from *S. europaea*, e.g., Szymańska et al. (2016) observed a higher frequency of endophytic bacteria *Serratia marcescens*, *Kushneria marisflavi*, *Microbacterium* sp., *Hymenobacter psychrotolerans* capable of IAA synthesis as compared to rhizosphere bacteria reflecting on direct interaction between the *S. europaea* and endophytes. The endophytic fungi from the roots of *S. europaea* synthesized more IAA compare to endophytes from the shoots (Furtado et al. 2019b).

Bacterial ACC deaminase (1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase) reduces plant ethylene levels and involved in nitrogen fixation, both are considered important mechanisms in bacteria that can promote plant growth, especially under stress conditions (del Carmen Orozco-Mosqueda et al. 2020). ACC deaminase-producing root endophytes isolated from *S. europaea*: *Pseudomonas* sp. I-S-E-12 and *Rhodococcus erythropolis* I-S-E-16 strains showed the presence of a gene encoding dinitrogenase reductase (Szymańska et al. 2016). Similarly, *S. europaea* endophytes with ACC deaminase activity belonging to the genera *Arthrobacter*, *Bacillus*, *Planococcus*, and *Variovorax* enhanced *in vitro* *S. europaea* seedlings growth in the presence of increasing concentrations of NaCl. In addition, these strains were also able to solubilize phosphate and produce IAA, which stimulated seed germination and increased the rate of root development (Zhao et al. 2016b).

Plant growth-promoting endophytes are able to release siderophores that are involved in biocontrol mechanisms thus preventing the growth of pathogenic microorganisms in plant hosts (Johnson 2008). High synthesis of siderophores was obtained for bacteria *Streptomyces* sp., *S. griseoplanus*, and *Bacillus thuringiensis* (Szymańska et al. 2016). Siderophores production by endophytic fungi was the most active function reported in *S. europaea* (Furtado et al. 2019b).

Hryniewicz et al. (2019) analyzed the endophytic diazotrophs of *S. europaea* which represented a broad range of N₂-fixing bacteria with Actinobacteria

dominating at the site characterized by higher salinity, and Proteobacteria at lower salinity. Most of the isolates from Actinobacteria belonged to the genus *Curtobacterium* (*Curtobacterium* sp., *C. flaccumfaciens*, *C. herbarum*) and *Microbacterium* (*Microbacterium* sp., *M. kitamiense*, *M. oxydans*), and some of them to *Rhodococcus*, *Mycobacterium*, *Cellulomonas*, *Sanguibacter*, *Clavibacter*, *Cryocola/Labedella*, *Frigoribacterium*, *Agreia*, *Herbiconiux*, and *Plantibacter*. The presence of the *nifH* gene was also identified in *S. europaea* endophytic bacteria: *Pseudomonas* sp. ISE12 and *R. erythropolis* ISE16 (Szymańska et al. 2016).

Phosphate solubilizers, e.g., *Bacillus endophyticus*, *B. tequilensis*, *Planococcus rifietoensis*, *Variovorax paradoxus*, and *Arthrobacter agilis* were identified in *S. europaea* roots (Zhao et al. 2016b). No activity for phosphate solubilization was observed in fungal endophytes of *S. europaea* (Furtado et al. 2019b).

All fungi possess pathways to biosynthesize polyamines, which are important in restoring cellular homeostasis under stressful conditions (Nikolaou et al. 2009). Furtado et al. (2019b) investigated the fungal culturable diversity and found nearly all of the strains possessed the ability to produce polyamines (90% of isolated strains from shoots and 83% from roots). However, the strains isolated from the *S. europaea* shoots actively produced polyamines that were correlated with the hyper-accumulation of salts in *Salicornia* shoots, which can be more stressful for fungal colonization.

The sulfur-oxidizing genera *Sulfurimonas* and *Halochromatium* were significantly abundant in the root endosphere and rhizosphere in *S. europaea* (Yamamoto et al. 2018). According to a previous report, *Sulfurimonas* is involved in host detoxification by oxidizing sulfide and producing sulfate as an end product, suggesting that the accumulation of these bacteria around the rhizosphere might be critical for the host tolerance of coastal environments (Fahimipour et al. 2017).

Most of the endophytic fungal strains isolated from two saline sites in Poland displayed proteolytic, lipolytic, and chitinolytic activity (Furtado et al. 2019b). On comparing the two sites, the fungal strains obtained from higher salinity site possessed higher cellulolytic, proteolytic, and amylolytic activities. While the strains isolated from lower salinity sites possessed proteolytic, lipolytic, and chitinolytic activities. Fungal strains *Aureobasidium pullulans* and *Sarocladium* sp. displayed high cellulolytic activity (Furtado et al. 2019a, b). Endophytic bacteria *Bacillus baekryungensis*, *Thalassospira permensis*, and *Xanthomonadales* sp. from the same sites showed high activity for hydrolysis of cellulose (Szymańska et al. 2016). High proteolytic activity was exhibited by few fungal strains, e.g., *Sarocladium* sp., *Stereum gausapatum*, *Epicoccum nigrum*, *Epicoccum* sp., *Porostereum spadiceum*, and *Stemphylium* sp. (Furtado et al. 2019b). Endophytic fungi belonging to *Aureobasidium* sp. tested positive for all the enzymatic activities, while some strains were negative for most of the activities, e.g., *Coprinellus domesticus*, *C. ellisii*, and *Arthrinium arundinis* (Furtado et al. 2019b).

Many pigmented spore- and/or mycelia-forming fungi black fungi or dematiaceous fungi such as the genera *Alternaria*, *Phoma*, *Cladosporium*, *Lewia*, *Pleospora*, *Epicoccum*, *Stemphylium*, *Ascochyta*, *Plenodomus*, *Neocamarosporium*, *Dematiopleospora*, *Aspergillus*, *Penicillium*, *Eurotium*, *Talaromyces*, *Fusarium*,

and *Aureobasidium* are frequently isolated from *S. europaea* in salt marsh and desert areas (Okane and Nakagiri 2015; Furtado et al. 2019b). This suggests that pigmented dematiaceous fungi universally inhabit this halophyte and can play an important ecological role in plant stress resistance as they possess some protective substances and are capable of tolerating extreme temperatures, desiccation, and saline environments (Gostinčar et al. 2009).

Endophytes can have myriad effects on host plant fitness, with the outcome of interactions ranging from beneficial to antagonistic. They exhibit a range of symbiotic relationships with their host plant and are well known to contribute to plant fitness, which helps the host to better adapt in stress conditions (Gopi and Jayaprakashvel 2017; Hryniewicz et al. 2019; del Carmen Orozco-Mosqueda et al. 2020). Therefore, the application of endophytes in crop productivity has gained importance today. Few studies on the compatibility and role of *S. europaea* endophytes have been established experimentally. For instance, five *S. europaea* strains, namely *Bacillus endophyticus*, *Bacillus tequilensis*, *Planococcus rifietoensis*, *Variovorax paradoxus*, and *Arthrobacter agilis* inoculated in *S. europaea* seeds significantly enhanced seed germination percentage, seedling growth, shoot and root length under salt stress condition (approx. 500 mM NaCl) (Zhao et al. 2016b). A pot experiment by Piernik and co-workers (2017) provided evidence that endophytic bacteria (*Xanthomonadales* sp. CSE-34 and *Pseudomonas* sp. ISE-12) isolated from *S. europaea* shoot and root stimulated the growth of *Beta vulgaris* (cv. Zentaur poly) under salinity conditions (approx. 300 mM NaCl). Szymańska et al. (2019) showed that halotolerant plant growth-promoting endophytic bacteria may have a beneficial effect on the growth and development of *Brassica napus* L. cultivated under salt stress conditions. *Pseudomonas stutzeri* ISE12 stimulated the elongation of roots, hypocotyls, and stems of *B. napus* and decreased the level of oxidative damage to cellular membranes exposed to salt stress. Another study re-inoculated *Staphylococcus* sp. (isolated from *S. europaea*) in *S. europaea* seedlings and found that this strain promoted plant growth and alleviated the negative effects of salinity at 200 mM NaCl in comparison to the non-inoculated plants (Komaresofla et al. 2019). Further, fungal endophytes isolated from *S. europaea* have also demonstrated plant growth-promoting effects in *Lolium perenne* (Furtado et al. 2019b). These fungal strains were selected based on specific properties (positive metabolic activity for IAA, siderophores, polyamines, and enzyme activity for cellulase, protease).

21.6 Conclusions

Through this chapter, we provide an overview of the community diversity and role of the closely associated microbiome, i.e., the endophytes present in halophyte *S. europaea*. The most abundant endophytic community among bacteria is the phylum Proteobacteria, while the majority of the fungal endophytes mainly belong to Ascomycota. Some of the factors responsible for shaping the endophytic diversity

in *S. europaea* include soil salinity, acclimatization time for microbes, halophytic plant species, plant organ, and stages of plant growth. The halophytic plant host, e.g., *S. europaea*, filters the specific microbiome. The saline soil harbors a higher microbial diversity in comparison to the endophytic diversity in roots (closer to the soil and the main entry point for endophytes), while the diversity in shoots is very low (the shoot accumulates salt that can be detrimental to the endophytes). Overall, we can infer that increasing salinization may eventually disturb the plant–endophytic association, regardless of the plant host having robust mechanisms to cope with salt stress. Moreover, much of the research on *S. europaea* endophytic microbiome found they are mainly halotolerant and possess traits that contribute toward the host and non-host plant growth and salt tolerance. Application of these endophytes for commercial use requires optimization of endophyte inocula, as questions on their association in natural field conditions, the effect of climate change, and the microbial diversity present in the new site that may affect the endophyte functioning in new host needs investigation.

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References

- Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. *Biol Control* 46:1
- Booth T, Gorrie S, Muhsin TM (1988) Life strategies among fungal assemblages on *Salicornia europaea* aggregate. *Mycologia* 80:176–191
- del Carmen O-MM, Glick BR, Santoyo G (2020) ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. *Microbiol Res* 235:126439
- Fahimipour AK, Kardish MR, Lang JM, Green JL, Eisen JA, Stachowicz JJ (2017) Global-scale structure of the eelgrass microbiome. *Appl Environ Microbiol* 83(12):e03391–e03316
- Fan C (2020) Genetic mechanisms of salt stress responses in halophytes. *Plant Signal Behav* 15(1):1704528
- Flowers TJ, Colmer TD (2015) Plant salt tolerance: adaptations in halophytes. *Ann Bot* 115:327–331
- Furtado BU, Gołębiewski M, Skorupa M, Hulisz P, Hryniewicz K (2019a) Exploring bacterial and fungal endophytic microbiomes of *Salicornia europaea*. *Appl Environ Microbiol* 85(13): e00305–e00319
- Furtado BU, Szymańska S, Hryniewicz K (2019b) A window into fungal endophytism in *Salicornia europaea*: deciphering fungal characteristics as plant growth promoting agents. *Plant Soil* 445(1–2):577–594
- Gopi K, Jayaprakashvel M (2017) Endophytic fungi from halophytes and their antioxidant potential. *Res J Pharm Technol* 10(11):4105–4107
- Gostinčar C, Grube M, De Hoog S, Zalar P, Gunde-Cimerman N (2009) Extremotolerance in fungi: evolution on the edge. *FEMS Microbiol Ecol* 71(1):2–11
- Hryniewicz K, Patz S, Ruppel S (2019) *Salicornia europaea* L. as an underutilized saline-tolerant plant inhabited by endophytic diazotrophs. *J Adv Res* 19:49–56
- Hyde KD, Soyong K (2008) The fungal endophyte dilemma. *Fungal Divers* 33:163–173
- Isca VMS, Seca AML, Pinto DCGA, Silva AMS (2014) An overview of *Salicornia* genus: the phytochemical and pharmacological profile. Research review, Natural products. Daya Publishing House, New Delhi, pp 145–164

- Johnson L (2008) Iron and siderophores in fungal–host interactions. *Mycol Res* 112:170–183
- Komaresofla BR, Alikhani HA, Etesami H, Khoshkholgh-Sima NA (2019) Improved growth and salinity tolerance of the halophyte *Salicornia* sp. by co-inoculation with endophytic and rhizosphere bacteria. *Appl Soil Ecol* 138:160–170
- Litalien A, Zeeb B (2019) Curing the earth: a review of anthropogenic soil salinization and plant-based strategies for sustainable mitigation. *Sci Total Environ* 698:134235
- Momonoki YS, Kamimura H (1994) Studies on the mechanism of salt tolerance in *Salicornia europaea* L.: I. Changes in pH and osmotic pressure in *Salicornia* plants during the growth period. *Jpn J Crop Sci* 63(3):518–523
- Muhsin TM, Booth T (1987) Fungi associated with halophytes of an inland salt marsh, Manitoba, Canada. *Can J Bot* 65:1137–1151
- Munns R, Passioura JB, Colmer TD, Byrt CS (2020) Osmotic adjustment and energy limitations to plant growth in saline soil. *New Phytol* 225(3):1091–1096
- Nikalje GC, Srivastava AK, Pandey GK, Suprasanna P (2018) Halophytes in biosaline agriculture: mechanism, utilization, and value addition. *Land Degrad Dev* 29(4):1081–1095
- Nikalje GC, Yadav K, Penna S (2019) Halophyte responses and tolerance to abiotic stresses. In: Hasanuzzaman M, Nahar K, Öztürk M (eds) *Ecophysiology, abiotic stress responses and utilization of halophytes*. Springer, Singapore, pp 1–23
- Nikolaou E, Agraftioti I, Stumpf M, Quinn J, Stansfield I, Brown AJ (2009) Phylogenetic diversity of stress signalling pathways in fungi. *BMC Evol Biol* 9(1):44
- Okane I, Nakagiri A (2015) Assemblages of endophytic fungi on *Salicornia europaea* disjunctively distributed in Japan: towards clarification of the ubiquity of fungal endophytes on halophytes and their ecological roles. *Curr Sci*:62–71
- Park JM, Park JH, Kim JG, You YH (2016) Securing and analysis of fungal endophytic diversity from roots of *Salicornia europaea* L. *J Life Sci* 50(3):89–98
- Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) *Microbial ecology of leaves*, Brock/Springer Series in Contemporary Bioscience. Springer, New York, NY
- Piernik A, Hryniewicz K, Wojciechowska A, Szymañska S, Lis MI, Muscolo A (2017) Effect of halotolerant endophytic bacteria isolated from *Salicornia europaea* L. on the growth of fodder beet (*Beta vulgaris* L.) under salt stress. *Arch Agron Soil Sci* 63(10):1404–1418
- Promptutha I, Hyde KD, McKenzie EH, Peberdy JF, Lumyong S (2010) Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? *Fungal Divers* 41(1):89–99
- Shahzad B, Fahad S, Tanveer M, Saud S, Khan IA (2019) Plant responses and tolerance to salt stress. In: *Approaches for enhancing abiotic stress tolerance in plants*. Taylor, Francis, pp 61–77
- Shi YW, Lou K, Li C, Wang L, Zhao ZY, Zhao S, Tian CY (2015) Illumina-based analysis of bacterial diversity related to halophytes *Salicornia europaea* and *Suaeda aralocaspica*. *J Microbiol* 53(10):678–685
- Singh D, Buhmann AK, Flowers TJ, Seal CE, Papenbrock J (2014) *Salicornia* as a crop plant in temperate regions: selection of genetically characterized ecotypes and optimization of their cultivation conditions. *AoB Plants*:6
- Slama I, Abdelly C, Bouchereau A, Flowers T, Saviour A (2015) Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann Bot* 115:433–447
- Sonjak S, Udovič M, Wraber T, Likar M, Regvar M (2009) Diversity of halophytes and identification of arbuscular mycorrhizal fungi colonising their roots in an abandoned and sustained part of Sečovlje salterns. *Soil Biol Biochem* 41(9):1847–1856
- Sultanpuram VR, Mothe T (2019) Microbial ecology of saline ecosystems. In: *Microorganisms in saline environments: strategies and functions*. Springer, Cham, pp 39–63
- Szymañska S, Piernik A, Baum C, Złoch M, Hryniewicz K (2014) Metabolic profiles of microorganisms associated with the halophyte *Salicornia europaea* in soils with different levels of salinity. *Ecoscience* 21(2):114–122

- Szymańska S, Płociniczak T, Piotrowska-Seget Z, Hryniewicz K (2016) Endophytic and rhizosphere bacteria associated with the roots of the halophyte *Salicornia europaea* L.–community structure and metabolic potential. *Microbiol Res* 192:37–51
- Szymańska S, Borroso L, Brusetti L, Hulisz P, Furtado B, Hryniewicz K (2018) Bacterial microbiome of root-associated endophytes of *Salicornia europaea* in correspondence to different levels of salinity. *Environ Sci Pollut Res* 25:25420–25431
- Szymańska S, Dąbrowska GB, Tyburski J, Niedojadło K, Piernik A, Hryniewicz K (2019) Boosting the *Brassica napus* L. tolerance to salinity by the halotolerant strain *Pseudomonas stutzeri* ISE12. *Environ Exp Bot* 163:55–68
- Tadych M, White JF, Moselio S (2009) Endophytic microbes. In: Schaechter M (ed) *Encyclopedia of microbiology*, pp 431–442
- Ushakova SA, Kovaleva NP, Gribovskaya IV, Dolgushev VA, Tikhomirova NA (2005) Effect of NaCl concentration on productivity and mineral composition of *Salicornia europaea* as a potential crop for utilization NaCl in LSS. *Adv Space Res* 36(7):1349–1353
- Ventura Y, Sagi M (2013) Halophyte crop cultivation: the case for *salicornia* and *sarcocornia*. *Environ Exp Bot* 92:144–153
- Yamamoto K, Shiwa Y, Ishige T, Sakamoto H, Tanaka K, Uchino M, Tanaka N, Oguri S, Saitoh H, Tsushima S (2018) Bacterial diversity associated with the rhizosphere and endosphere of two halophytes: *Glax maritima* and *Salicornia europaea*. *Front Microbiol* 9:2878
- You Y, Lee M, Kim J (2014) Endophytic fungal diversity isolated from the root of halophytes in Taean peninsula. *Korean J Mycol* 42:269–275
- Zhao S, Zhou N, Zhao ZY, Zhang K, Tian CY (2016a) High-throughput sequencing analysis of the endophytic bacterial diversity and dynamics in roots of the halophyte *Salicornia europaea*. *Curr Microbiol* 72(5):557–562
- Zhao S, Zhou N, Zhao ZY, Zhang K, Wu GH, Tian CY (2016b) Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. *Curr Microbiol* 73(4):574–581