Chapter 8
Enterobacter: Role in Plant Growth Promotion

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8.1 Introduction

Of all the variables that impact upon plant growth, soil microbial activity is arguably the very complex but plays a very important role in agricultural (or conservation) management. The importance of the microbiota to biogeochemistry has long been appreciated (Conrad 1996). Interactions between plants and microbes have long been known and we are increasingly aware of inter-kingdom communication signals across a broader range of ecological interactions than simple two-species mutualisms. Few would argue the point that the microbiota are an intimate part of the plant ecosystem and that understanding their roles will lead to new management opportunities. By describing patterns of variation in soil microbiota and explaining the basis of their ecological interactions with plants, soil microbial ecologists aim to develop new management tools for plant systems.

Plant growth promoting rhizobacteria (PGPR) can have an impact on plant growth and development in two different ways: indirectly or directly. The indirect promotion of plant growth occurs when bacteria decrease or prevent some of the deleterious effects of a phytopathogenic organism by one or more mechanisms. On the other hand, the direct promotion of plant growth by PGPR generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment (Glick 1995). Rhizosphere bacteria multiply to high densities on plant root surfaces where root exudates and root cell lysates provide the ample nutrients. Sometimes, they exceed 100 times to

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those densities found in the bulk soil (Campbell and Greaves 1990). Certain strains of these plant-associated bacteria stimulate plant growth in multiple ways: (1) they may fix atmospheric nitrogen, (2) reduce toxic compounds, (3) synthesize phytohormones and siderophores, or (4) suppress pathogenic organisms (Bloemberg and Lugtenberg 2001). Research on the "biocontrol" activity of rhizobacteria has seen considerable progress in the recent years. Disease suppression of soilborne pathogens includes competition for nutrients and production of antimicrobial compounds or lytic enzymes for fungal cell walls or nematode structures (Persello-Cartieaux 2003). By contrast, systemic resistance can also be induced by rhizosphere-colonizing *Pseudomonas* and *Bacillus* species where the inducing bacteria and the challenging pathogen remained spatially separated excluding direct interactions (Ryu et al. 2004).

Family Enterobacteriaceae, in the class Gammaproteobacteria, encompass a wide range of microorganisms including 42 genera in the last edition of Bergey's Manual of Systematic Bacteriology (Garrity 2005), but half of the isolates of new or unusual Enterobacteriaceae seem to be misidentified (Farmer 2005). To avoid misidentification of species in future, Paradis et al. (2005) propose the classification of species within Enterobacteriaceae by studying the genes encoding the elongation factor Tu and F-ATPase-β-subunit additionally to the gene encoding the 16S small ribosomal subunit. The genera within the family Enterobacteriaceae that feature members described as plant growth promoting bacteria (PGPB) are *Citrobacter*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Kluyvera*, *Pantoea* and *Serratia*, although some of these genera also contain species reported to be plant pathogens, for example *Erwinia carotovora* (Rodríguez-Díaz et al. 2008). The seven genera mentioned above have undergone changes in their taxonomy in the time elapsed between the two most recent releases of Bergey's Manual of Systematic Bacteriology. The genus *Enterobacter* was first described by Hormaeche and Edwards in 1960 (Hormaeche and Edwards 1960). Since *Enterobacter aerogenes* is considered a homotypic synonym to *Klebsiella mobilis* and *Enterobacter agglomerans* is transferred to the genus *Pantoea*, 13 species are left in the genus *Enterobacter*, i.e., *Enterobacter amnigenus*, *Enterobacter cowanii*, *Enterobacter gergoviae*, *Enterobacter intermedius*, *Enterobacter pyrinus*, *Enterobacter sakazakii*, and seven species which are combined in the so-called *Enterobacter cloacae* complex which include *Enterobacter asburiae*, *E. cloacae*, *E. dissolvens*, *E. hormaechei*, *E. kobei*, *E. nimipressuralis*, and *E. cancerogenus*, the senior synonym of *E. taylorae*. This genus contains the *Enterobacter agglomerans* group, which was extremely heterogeneous. Strains previously included in the *E. agglomerans* group have been proposed to be relocated into the genera *Erwinia*, *Leclercia* and *Pantoea*. Another species, *E. intermedius*, was first described as a senior subjective synonym for the species *Kluyvera cochleae* as shown by DNA–DNA hybridization (Brenner and Farmer 2005) but was later transferred to the genus *Kluyvera* as the species *K. intermedia* comb. nov. and *K. cochleae* was demonstrated to be a later synonym of *K. intermedia* (Pavan et al. 2005). PGPB strains must be rhizospheric competent and are able to survive and colonize in the rhizospheric soil (Cattelan et al. 1999). Unfortunately, the interaction between associative PGPR and plants can be

unstable. The good results obtained in vitro cannot always be dependably reproduced under field conditions (Chanway and Holl 1993; Zhender et al. 1999). The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects adversely (sometimes) on plant. The role of environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil can not be ruled out. To achieve the maximum growth promoting interaction between PGPR and nursery seedlings, it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are altered by various environmental factors, including the presence of other microorganisms (Bent et al. 2001). Therefore, it is necessary to develop efficient strains suitable for field conditions. One possible approach is to explore soil microbial diversity for PGPR having combination of plant growth promoting (PGP) traits and well adapted to particular soil environment. Ahmad and Khan (2010) studied *Enterobacter* for fungicide tolerance and production of PGP traits both in the presence and absence of fungicides. Strain PS2 showed PGP activities even in the presence of fungicides which, however, decreased progressively corresponding with the increase in fungicide concentration. Keeping in view the above constrains, the present chapter was designed to screen certain rhizospheric bacterial isolates belonging to the genera *Enterobacter* for their multiple PGP activities. Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals. We observed few rhizobacteria tolerant to heavy metals and exhibiting a couple of PGP activities. It was also apparent that more cultures of PGPR isolated from chickpea rhizosphere were tolerant to elevated levels of heavy metals. Burd et al. (1998) found that by decreasing heavy metal toxicity, PGPR increase plant growth. The selection of microorganisms both metal tolerant and efficient in producing PGP compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils (Carlot et al. 2002). Heavy metals, at higher concentration, are toxic to cells and may cause cell death by interacting with nucleic acids and enzyme active sites (Ohsumi et al. 1988; Hazel and Williams 1990; Cervantes and Gutierrez-Corana 1994). On the otherhand, *Azotobacter* spp, when inoculated into heavy metal contaminated soil, inhibited N_2 fixation (Briely and Thornton 1983). Chromium-resistant *Pseudomonads*, isolated from paint industry effluents, were able to stimulate seed germination and growth of *Triticum aestivum* in the presence of potassium dichromate (Hasnain and Sabri 1996). It is expected that inoculation with rhizobacteria containing PGP characteristics consequently promote root and shoot growth as well as nodulation. Application of organic acid secreting bacteria for counteracting the toxicity of metal ions to their plant hosts has been developed. *E. asburiae* PS13 isolates from pigeon pea rhizosphere (Gyaneshwar et al. 1999) is known to produce different low molecular weight organic acid depending on the carbon source available. Kavita et al. (2008) reported that mung bean seedlings inoculated with *E. asburiae* PS13, a gluconic acid producer, enhanced plant growth in the presence of phytotoxic levels of Cd^{2+} in gnotobiotic pot experiments as compared to the uninoculated Cd-treated plants. Addition of organic acids to Cd-stressed seedlings

promoted root elongation. Deepa et al. (2010) studied PGP potential of strains NII-0907(*E. aerogenes*), NII-0929 (*E. aerogenes*), NII-0931(*E. cloacea*) and NII-0934 (*E. asburiae*) members of the genus *Enterobacter*. All the four enterobacter species are very good phosphate solubilizers (60.1–79.5 μg/ml/day after tenth day of incubation); indole acetic acid (IAA) producers (23.8–104.8 μg/ml/day after 48 h of incubation); HCN producers and siderophore producers. Studies have been carried out to investigate their influence on cowpea and recorded significant increase in higher root and shoot lengths compared with uninoculated control. Further evaluation of the isolates exhibiting multiple PGP traits on soil–plant system is needed to uncover their efficacy as effective PGPR.

8.2 Plant Growth Promoting Attributes

Enterobacter have the potential to contribute in the development of sustainable agricultural systems. Generally, *Enterobacter* function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and lessening or preventing the plants from diseases. The mechanisms of PGPR-mediated enhancement of plant growth and yield of many crops are not yet fully understood. However, the possible explanation include (a) the ability to produce a vital enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene in the root of developing plants thereby increasing the root length and growth; (b) the ability to produce hormones such as auxin, i.e., IAA; (c) a symbiotic nitrogen fixation; (d) antagonism against phytopathogenic bacteria by producing siderophores; and (e) solubilization and mineralization of nutrients, particularly mineral phosphates.

8.2.1 Nitrogen Fixation

Atmospheric N constitutes approximately 80% of the air we breathe. Although abundant and ubiquitous in the air, N is the most limiting nutrient to plant growth because the atmospheric N is not available for plant uptake. Some bacteria are capable of N_2 fixation from the atmospheric N pool. These bacteria form various associations with plants: (a) Many free-living N_2-fixing bacteria occur in soil. (b) Some have adapted to form symbioses; others have intimate endophytic associations with plants. (c) Few live in close association in the plant root zone (rhizosphere) without forming intimate endophytic symbioses. Infact, the amount of N fixed by these different systems is considerable, although variation resulting from environmental conditions or different plant–microbe combinations is vast. The close proximity of these microorganisms to their host plants allows efficient plant use of fixed N and minimizes volatilization, leaching, and denitrification. In

addition to symbiotic bacteria infecting roots, numerous taxa of less intimately associated N_2-fixing bacteria can be considered for crop growth improvement. Examples of such bacteria include *Acetobacter diazotrophicus* and *Herbaspirillum* spp. associated with sugarcane, sorghum, and maize (Triplett 1996; James et al. 1997; Boddey et al. 2000), *Azoarcus* spp. associated with kallar grass (*Leptochloa fusca*) (Malik et al. 1997), and *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Rhizobium* associated with rice and maize (James 2000).

8.2.2 Siderophore Production

Siderophores are low molecular weight bio-molecules secreted by micro-organisms in response to iron starvation for acquisition of iron from insoluble forms by mineralization and sequestration (Lankford 1973). Although some siderophores are known to chelate other ions, their specificity for iron is the most consistent feature. Lost ability to synthesize siderophores leads to loss of ability to synthesize cyanide and thus marred biocontrol abilities of strain proves the physiological importance of iron (Voisard et al. 1989). Siderophores produced by rhizosphere inhabitants have been studied well and it has been reported that the ability to produce siderophores not only improves rhizosphere colonization of producer strain but also plays an important role in iron nutrition of plant (Vansuy et al. 2007) and antagonism against phytopathogens (Chincholkar et al. 2007; Singh et al. 2008, 2010). Role of siderophores in induced systemic resistance (ISR) in plants was also well appreciated (De Meyer et al. 1999). The presence of an efficient iron uptake system can therefore contribute to protect the host plant against pathogenic infections. *E. coli* K12, *Enterobacter* sp. 638 (Taghavi et al. 2010) are able to synthesize the siderophore enterobactin (EntD, EntF, EntC, EntE, EntB and EntA), secrete (EntS), recover the iron–enterobactin complex using a ferric siderophore uptake system (ExbDB), and extract the iron using an enterobactin esterase (Fes) after internalization of the iron–enterobactin complex. The genes involved in this biosynthesis of enterobactin are grouped with genes encoding two ABC transporters involved in iron uptake (sitABCD and fepCGDB) in a large cluster of 17 genes (Ent638_1111-1128).

8.2.3 1-Aminocyclopropane-1-Carboxylase

ACC deaminase is a multimeric enzyme with a monomeric subunit molecular mass of approximately 35–42 kDa. It is a sulfhydryl enzyme that utilizes pyridoxal 5-phosphate as an essential co-factor. Pyridoxal phosphate is tightly bound to the enzyme in the amount of approximately one molecule per subunit; it displays a characteristic pyridoxaldimine visible absorbance at 418 nm (Jackson 1997). In this case, the substrate, ACC is exuded by plant tissues and is then taken up by the

ACC deaminase-containing microbe (Saraf et al. 2010). There is a wide range (>100-fold) in the level of ACC deaminase activity that is observed in nature from one organism to another so that these organisms may be conceptually divided into two groups: those with either high or low enzyme activity. High ACC deaminase expressing organisms typically bind relatively nonspecifically to a variety of plant surfaces. This group includes most, if not all, rhizosphere and phyllosphere organisms as well as endophytes, all of which can act as a sink for ACC produced as a consequence of plant stress. These organisms display little preference for one particular plant over another. On the other hand, low deaminase-expressing organisms bind only to specific plants or are expressed only in certain tissues, and they do not lower the overall level of ethylene in the plant, but rather prevent a localized rise in ethylene levels. This group includes most, if not all, rhizobia as well as plant ACC deaminases (Glick 1995). Burd et al. (2000) reported on the potential of the ACC deaminase-producing bacterium *Kluyvera ascorbata* SUD165 to protect canola (*Brassica napus*) and tomato (*Lycopersicon esculentum*) seeds from the heavy metal toxicity induced by high concentrations of nickel (Ni), lead (Pb), and zinc (Zn). Saravanakumar and Samiyappan (2007) reported that *Pseudomonas fluorescens* TDK1 possessing ACC deaminase activity enhanced the soil salinity resistance of groundnuts and observed increase yields over the groundnuts treated by *Pseudomonas* spp. that lacked ACC deaminase activity. However, the role of ACC deaminase in root colonization in bacteria is unknown (Wang et al. 2002). ACC deaminase from bacteria, *Pseudomonas putida* and *P. fluorescens*, *Enterobacter cloacae* CAL2 and UW4 (Shah et al. 1998), *Kluyvera ascorbata* SUD165 (Burd et al. 1998), and yeast, *Hansenula saturnus* (Honma and Shimomura 1978), and fungus, *Penicillium citrinum* (Jia et al. 2006) have been reported. However, work in this area represents only plant–microbe interaction.

8.2.4 Antimicrobial Compounds

Considerable progress has been made over the past two decades to elucidate the mechanisms by which PGPR suppress diseases. The primary mechanism of biocontrol by fluorescent *pseudomonads* involves production of antibiotics such as 2,4-diacetylphloroglucinol (PHL) (Bangera and Thomashaw 1996), pyoluteorin (PLT), pyrrolnitrin (PRN), phenazine-1-carboxyclic acid (PCA), 2-hydroxy phenazines and phenazine-1-carboxamide (PCN). In addition to direct antipathogenic action, antibiotics also serve as determinants in triggering ISR in the plant system and contribute to disease suppression by conferring a competitive advantage to biocontrol agents (Fravel 1988). Synergism between antibiotics and ISR may further increase host resistance to plant pathogens. Some other antibiotics produced by PGPR include pyrrolnitrin, oomycinA, viscosinamide, butyrolactones, kanosamine, zwittermycin-A, aerugine, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antitumor antibiotics FR901463, cepafungins (Constantinescu 2001), and antiviral antibiotic karalicin. These antibiotics are

known to possess antiviral, antimicrobial, insect and mammalian antifeedant, antihelminthic, phytotoxic, antioxidant, cytotoxic, antitumour, and PGP activities. The *Enterobacter* sp. 638 genome (Taghavi et al. 2010) encodes a chloramphenicol acetyltransferase (cat, Ent638_1533) which provides resistance to 20 mg/ml chloramphenicol. A precursor of the important electron carrier ubiquinone, 4-hydroxybenzoate, is also known to have antimicrobial activity. In contrast to *E. coli* K12, which is not able to degrade chorismate into 4-hydroxybenzoate and pyruvate, *Enterobacter* sp. 638 possesses the ubiC (Ent638_0243) gene that codes for the putative enzyme able to perform this reaction (Siebert et al. 1996).

8.2.5 Hormonal Signals Involved in Plant Growth Promotion

Most commonly proposed signal molecules for PGP include bacterial synthesis of the auxin, cytokinin, gibberellin, and breakdown of plant produced ethylene by PGPR mediated of 1-aminocyclopropane-1-carboxylate deaminase. In the last 5 years, additional signals from microbes have been found to play a role in plant morphogenetic processes, including the *N*-acyl-L-homoserine lactones (AHLs) and volatile organic compounds (VOCs). AHLs belong to a class of bacterial quorum sensing signals from Gram-negative bacteria. These compounds enable bacterial cells to regulate gene expression depending on population density. Very recently, it was found that AHLs can be recognized by plants, alter gene expression in roots and shoots, and modulate defense and cell growth responses. Similarly, bacterial volatiles such as acetoin and 2,3-butanediol produced by certain PGPR can be used for plant–bacteria communication and as a PGP triggers. Some rhizobacteria, such as strains from *B. subtilis*, *B. amyloliquefaciens*, and *Enterobacter cloacae*, promote plant growth by releasing volatiles (Ryu et al. 2003). The highest level of growth promotion was observed with 2,3-butanediol and acetoin. Mutants of *B. amyloliquefaciens* IN937a and *B. subtilis* GB03, blocked in the biosynthesis of these compounds, are inactive in plant growth promotion. More recently, Zhang et al. (2008) observed that *B. subtilis* GB03 increases the photosynthetic efficiency and chlorophyll content of *A. thaliana* through the modulation of endogenous signaling of glucose and abscisic acid sensing; thus the bacterium plays a regulatory role in the acquisition of energy by the plant.

8.3 Molecular Biology and General Features *Enterobacter* sp.

The genome of the gamma-proteobacterium *Enterobacter* sp. 638 (Taghavi et al. 2010), a member of the Enterobacteriaceae, is composed of a single circular chromosome of 4,518,712 bp with an overall G + C content of 52.98%, and of a 157,749 bp plasmid pENT638-1 having an overall G + C content of 50.57%. The chromosome of *Enterobacter* sp. 638 displays a clear GC skew transition, which corresponds with its replication origin (oriC) and terminus. Similar to *E. coli* K12, the oriC site contains a perfect DnaA-binding box (TTATCCACA) (Weigel et al. 1997), which is located

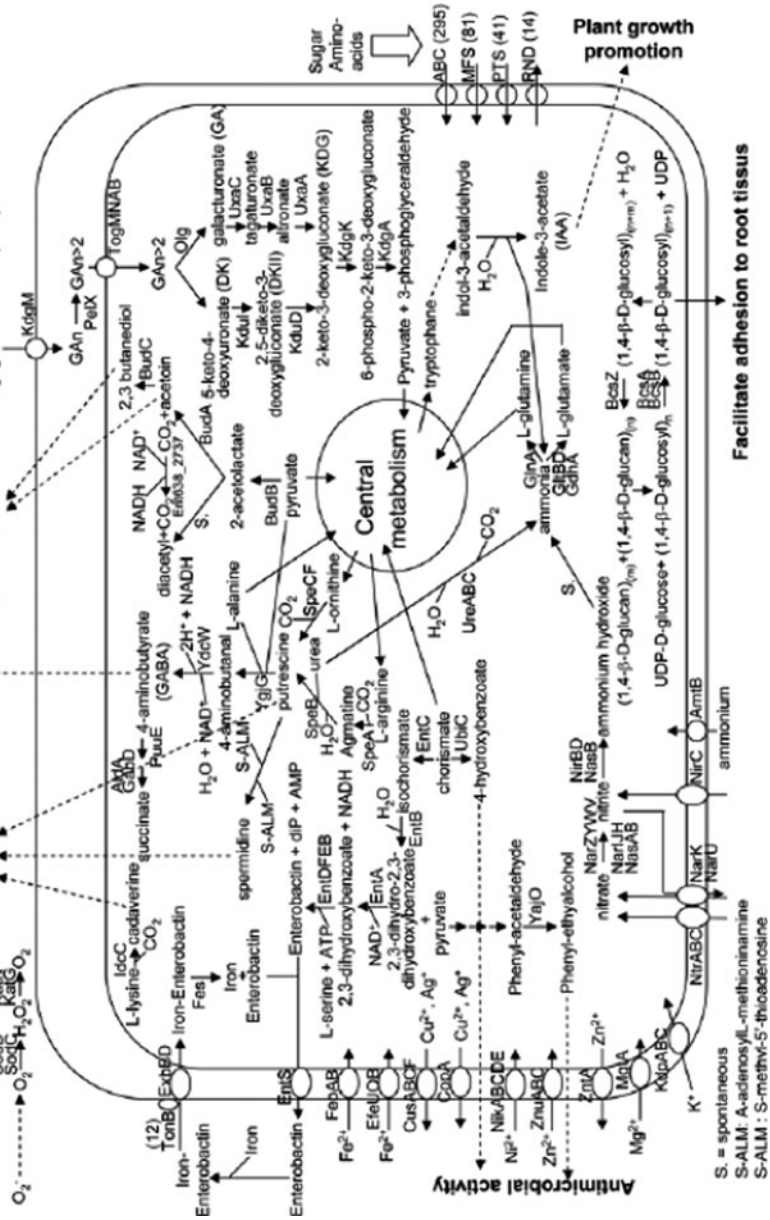

Fig. 8.1 Modified figure adapted from Taghavi et al. (2010). Overview of metabolism and transport in *Enterobacter* sp. 638. Predicted pathways for the interaction between *Enterobacter* sp. 638 and the plant are shown. Export or import of solutes is designated by the direction of the *arrow* through the transporter. The *dash arrows* show the putative function of certain molecular biosynthesized by *Enterobacter* sp. 638 in regard to its plant

31,985 bp upstream of the dnaA ATG start codon (at coordinate 4,487,245 bp). The *Enterobacter* sp. 638 genome encodes 4396 putative coding sequences (CDS), with 4247 CDS encoded on the chromosome representing a coding density of 87.9%. Plasmid pENT638-1 encodes 149 putative CDS having a coding density of 80.4%. After their manual annotation, 3561 CDS (81%) could be assigned to putative biological functions, while 836 CDS (19%) were annotated as hypothetical proteins of unknown function. For the CDS with unassigned functions, conserved hypothetical proteins are represented by 689 CDS (15.7%), while 147 CDS (3.3%) had no homology to any previously reported sequence. Using the COGnitor module from the MaGe system, 3597 CDS (81.8%) could be assigned to one or more COG functional classes. The repartition of *Enterobacter* sp. 638 CDS among the different COG classes is very similar to what is observed for *E. coli* K12 (Blattner et al. 1997). The three most abundant classes are amino acid (E), carbohydrate (G), and inorganic iron (P) transport and metabolism and represent more than 37% of all CDS, pointing to the symbiotic life styles of *Enterobacter* sp. 638 and *E. coli* K12 that require efficient uptake of host-provided nutrients. Seven sets of 5S, 16S, 23S rRNA genes, and one additional 5S rRNA gene were found. A total of 83 tRNA genes with specificities for all 20 amino acids and a single tRNA for selenocysteine have been identified. Based on the genome analysis, *Enterobacter* sp. 638 seems well adapted to survive in the plant rhizosphere because it encodes many transporters involved in carbohydrate, amino-acids, and iron uptake, as well as some heavy metal resistance genes. An overview of the metabolic properties and important transport pathways for interactions between *Enterobacter* sp. 638 and its plant host is presented in Fig. 8.1.

8.4 Selected Species of *Enterobacter*

The concept of introducing PGPR into the rhizosphere using the transplant plug is based on the hypothesis that their establishment in the relatively clean environment of planting media would afford them an opportunity to develop stable populations in the seedling rhizosphere, and that these populations would then persist in the field. This review covers the perspective of few selected species of *Enterobacter* and the role they are playing in plant growth promotion via direct and indirect mechanisms. The further elucidation of different mechanisms involved will help to make these bacteria a valuable partner in future agriculture.

8.4.1 Enterobacter cloacae UW5: Aromatic Amino Acid-Dependent Expression of Indole-3-Pyruvate Decarboxylase and Overexpression of hns Gene

Paradoxically, IAA produced by PGPR has been found to enhance host root system development. Plant roots colonize with the PGPR species *Azospirillum brasilense*

Sp6, *Enterobacter cloacae* UW5, and *Pseudomonas putida* GR12-2 displayed increase in root hair formation, the number and length of lateral roots, and/or primary root length that are dependent on bacterial IAA production.

A number of IAA biosynthetic pathways have been identified in bacteria, most requiring tryptophan as a precursor. Synthesis via the intermediates indole-3-acetamide or indole-3-pyruvate is widespread among IAA-producing bacteria. Most phytopathogens, such as *Agrobacterium tumefaciens* and *P. syringae* pv. *savastonoi*, use the indole-3-acetamide pathway to synthesize IAA (32, 54), while the indole-3-pyruvate pathway is found in many PGPR species, including *A. brasilense* and *E. cloacae*, and in the nonpathogenic epiphytic bacterium *Erwinia herbicola* 299R (Brandl and Lindow 1996; Costacurta et al. 1994). In the latter pathway, the precursor tryptophan is converted to indole-3-pyruvate by tryptophan transaminase, and indole-3-pyruvate is then converted to indole-3-acetaldehyde by indole-3-pyruvate decarboxylase (IPDC). IAA is produced after oxidation of indole-3-acetaldehyde by indole-3-acetaldehyde oxidase. The key enzyme in this pathway, IPDC, is encoded by *ipdC*, and elimination of *ipdC* abolishes IAA biosynthesis in *E. cloacae* UW5 and greatly reduces IAA production in *A. brasilense* and *E. herbicola* 299R (Brandl and Lindow 1996; Costacurta et al. 1994).

The activity of *tyrR* in *E. cloacae* UW5 was abolished by insertion of a tetracycline resistant cassette into the coding sequence, creating *E. cloacae* J35. The 2.1-kb tetracycline resistance cassette was amplified using tet-KpnI F and R primers and pJP2 as the template for PCR and then subcloned into pGEM-T Easy. A 1,177-bp *tyrR* fragment, amplified using the primers T1F and T4R, was also subcloned into pGEM-T Easy. The tetracycline resistance cassette was excised from pGEM-T Easy and inserted into a native KpnI site in *tyrR* (708 bp downstream from translation start codon) as a KpnI fragment. The interrupted *tyrR* gene fragment was excised from pGEM-T Easy and cloned into the NotI site in the suicide plasmid pJQ200SK, creating pJQ200TM. pJQ200TM was transformed into calcium chloride-competent *E. coli* S17-1 (*_pir*) cells and subsequently introduced into *E. cloacae* UW5 by conjugation. Double recombinants were identified by tetracycline resistance and gentamicin sensitivity. The site of insertion in the genome was verified by PCR amplification using the primers T1F and T1R (note that the T1R sequence is not present in pJQ200TM). The PCR amplicon generated from the genome of the *tyrR* insertional mutant (*E. cloacae* J35) was 2.1 kb larger than that from the wild-type strain, confirming the replacement of wild-type *tyrR* with the mutant *tyrR* gene fragment. Ryu and Patten (2008) described here that the transcription factor TyrR directly and positively controls *ipdC* expression and IAA production in the PGPR *E. cloacae* UW5 and that TyrR-dependent expression increases in response to exogenous tryptophan. A sequence with only a single base mismatch to the consensus sequence for the TyrR box (TGTAAA-N6-TTTACA) in *E. coli* (Pittard et al. 2005) has been identified in the promoter region of *ipdC* in this bacterium and in other closely related bacteria, even though the promoter sequences are otherwise quite dissimilar. Loss of IAA production and lower levels of *ipdC* expression following disruption of TyrR function through

insertional mutagenesis in mutant strain *E. cloacae* J35 confirmed the requirement for TyrR. The high degree of nucleotide sequence identity to the consensus sequence for the TyrR binding site suggests a strong TyrR protein-promoter DNA interaction. This is supported by the ability of purified TyrR to bind to the *ipdC* promoter fragment containing the TyrR box in vitro, and by the induction of *ipdC* expression, in the absence of an effector molecule. The observed transcription of *ipdC* in the absence of tryptophan supplements to the culture medium may be mediated by binding of endogenous aromatic amino acid cofactors to TyrR. Although TyrR can bind to strong boxes in the absence of cofactors, the addition of aromatic amino acids strengthens the interaction between TyrR and its recognition sequence (Andrews et al. 1991; Pittard et al. 2005). The increased affinity of TyrR for the promoter results in increased transcription, as was observed here by an increase in *ipdC* transcript abundance measured by real-time qRT-PCR and by an increase in *ipdC* promoter driven glucuronidase activity after addition of the TyrR cofactor tryptophan. An *E. cloaca* is an enteric, plant beneficial bacterium that suppresses damping off caused by *Pythium ultimum* on cucumber and other crops. An *Enterobacter cloaca* is a common seed associated bacterium that suppressed seed infections, protecting a number of plant species from *P. ultimum* induced damping off. Plant protection by *E. cloacae* is achieved largely by degradation of long chain unsaturated fatty acids in seed exudates, which eliminates the stimulation of *P. ultimum* sporangia. *E. cloacae* suppresses *P. ultimum* infections when applied as a seed coating of carrot, cotton, cucumber, lettuce, radish, sunflower, tomato, and wheat (Windstam and Nelson 2008).

Cloning and sequencing of the PCR fragment revealed that the transposon was inserted in the 5′ flanking region of a gene with homology to the *hns* gene encoding the small histone-like protein H-NS in *E. coli* and *Enterobacter* spp. (English et al. 2010) H-NS binds predominantly to AT-rich sequences, regions of intrinsic curvature commonly found in promoter sequences, and represses transcription of a large number of genes (Lucchini et al. 2006; Navarre et al. 2006). The expression of 5% of genes in *E. coli* is regulated by H-NS (Hommais et al. 2001), and many of these are important for adaptation to environmental changes such as alterations in temperature, pH, osmolarity, and growth phase (Stella et al. 2006). H-NS has also been noticed to repress genes that have been acquired by bacteria through horizontal gene transfer (Lucchini et al. 2006; Navarre et al. 2006). In addition to its role as a transcriptional repressor, H-NS positively regulates the production of flagellin and several outer membrane porins, lipopolysaccharide biosynthesis, and motility (Ono et al. 2005). Motility is important for plant colonization and conditions in the rhizosphere have been shown to select for motile strains (Martinez-Granero et al. 2006). Production of flagella and lipopolysaccharides has previously been shown to be required for plant colonization (Turnbull et al. 2001); therefore, it may be through the enhancement of one or more of these mechanisms that H-NS over expression in *E. cloacae* J28 promotes colonization of roots. Understanding the role of H-NS in regulating colonization has important implications for the development of robust PGPR strains for agricultural applications. It may be possible through

genetic engineering to enhance the ability of introduced inoculants to competitively colonize the root of crop plants.

8.4.2 Enterobacter ludwigii *sp.: A Novel Species of Plant Growth and Its Clinical Relevance*

This description is based on phylogenetic analyses of partial *hsp60* sequence data collected in a recent population genetic study (Hoffmann and Roggenkamp 2003) as well as on DNA–DNA-hybridization assays and phenotypic characterization performed in the frame of the present study. Phenotypic characterization was performed using API20E, Biotype 100, and a series of conventional tests. *E. ludwigii* strains are Gram-negative rods, motile, catalase positive, oxidase and DNAase negative, fermentative, and non-pigmented in nature. They exhibit the general characteristics of the family Enterobacteriaceae, the genus *Enterobacter*, and the *E. cloacae* complex. Growth occurs after 18–24 h at 15–42°C with an optimum at 36°C on all non-selective media such as Columbia agar with 5% sheep-blood, chocolate, TSA, Luria, or Brain–Heart agar as well as on semi-selective media such as MacConkey or ENDO agar in the form of non-pigmented colonies (Shoebitz et al. 2009).

A detailed biochemical profiling of the species is given in Table 8.1 showing some tests in use for differentiating *E. ludwigii* from the other species of the genus. Its identification is mainly possible by the ability to grow on 3-methyl-D-gluco-pyranose and on myo-inositol. All strains analysed produced a Bush class 1 beta-lactamase rendering resistance to ampicillin, amoxicillin plus clavulanic acid, and cefoxitin in the disk diffusion tests. About 20% of the strains displayed a resistance pattern typical for AmpC hyperproduction (resistance to piperacillin, piperacillin plus tazobactam, cefoxitin, cefotaxime, ceftazidime, and susceptibility to cefepime). All strains are susceptible to trimethoprim plus sulphamethoxazole, gentamicin, meropenem, and ciprofloxacin.

It is widely known that a variety of bacterial species, including PGPRs, can act as biocontrol agents protecting plants from bacterial and fungal diseases (Romero et al. 2007). *P. agglomerans*, a strain closely related to *E. ludwigii* as shown by its 16S rDNA sequence, is able to antagonize *Sclerotinia sclerotiorum* (Berg et al. 2002). To assess whether the strain has the capability to act as a biocontrol agent, antagonism towards *Fusarium solani* has been investigated. BNM 0357 strain depicted fungicidal activity on *F. solani* vegetative growth in mixed cultures as well as in cultures in which BNM 0357 and *F. solani* are not in contact. This behavior suggests that bacteria compete with fungal development (e.g., for nutrients in the culture) and also that a diffusible compound could be responsible for the fungal growth inhibition. As expected, *A. brasilense* showed no fungal inhibiting activity. *F. solani* spore germination was also inhibited by strain BNM 035. These results indicate that *E. ludwigii* BNM 0357 is an efficient fungal

Table 8.1 Differentiation of *Enterobacter* species

Species	Biochemical test[a]													
	D-fucose	L-fucose	α-D-melibiose	1-0-methyl-α-galacto-pyranoside	Esculin	α-L-rhamnose	D-arabitol	Dulcitol	Myo-inositol	Adonitol	3-0-Methyl-D-gluco-pyranose	Putrescine	3-Hydroxy-butyrate	Mucate
E. ludwigi sp. nov. cluster V	−	V	+	+	V	+	−	−	+	−	+	−	+	+
Type strain EN-119^T	−	−	+	+	+	+	−	−	+	−	+	−	+	+
E. absuriae	−	−	+ −	+ −	+	+ −		−	+	−	−	− +	+	V
E. kobei	−	−	+	+	−	+	−	V	+	−	−	V	+ −	V
E. hormaechie	V	+	− +	−	−	+	− +	+	−	−	V	−	−	V
E. cloacae	−	−	+	+	−	+	−	−	+	−	−	+	+	V
E. dissolvens	V	V	+	+	+	+	−	−	+	V	−	+	+	+
E. cancerogenus	+[f]	+[e]	−[b]	−[f]	+[b]	+[e]	−[e]	−[b]	−[e]	−[e]	+[e]	+[e]	−[e]	+[e]
E. nimipresuurallius	−[f]	−[e]	+[b]	−[f]	+[b]	+[e]	−[e]	−[b]	−[e]	−[e]	−[f]	−[e]	−[e]	+[e]
E. amnigenus	−[f]	−[e]	+[b]	+[f]	+[b]	+[e]	−[e]	-[b]	−[e]	−[e]	−[f]	−[e]	−[e]	+[e]
E. cowanii	−[c]	−[c]	+[c]	+[f]	+[c]	+[c]	−[c]	+[c]	−[c]	−[c]	−[f]	−[f]	−[f]	+[c]
E. georgoviae	−[f]	V[e]	+[b]	+[f]	+[b]	+[e]	+[e]	−[b]	V[e]	−[e]	−[f]	V[e]	V[e]	−[e]
E. intermedius	−[f]	V[e]	+[b]	+[f]	+[b]	+[e]	−[e]	+[b]	−[e]	−[e]	−[f]	−[e]	−[e]	+[e]
E. pyrinus	−[f]	−[e]	+[d]	−[f]	+[d]	+[d]	+[e]	−[d]	+[d]	−[d]	−[f]	+[f]	−[f]	−[e]
E. sakazakii	−[f]	−[e]	+[b]	+[f]	+[b]	+[e]	−[e]	−[b]	V[e]	−[e]	−[f]	+[e]	−[e]	−[e]

Table adapted from Hoffmanna et al. (2005)

[a]Incubation at 36°C. Symbols: −, 0–10%; − +, 10–20%; V, 20–80%; + −, 80–90%; +, 90–100%

[b]Sources of data according to O'Hara et al. (1989)

[c]Sources of data according to Inoue et al. (2000)

[d]Sources of data according to Chung et al. (1993)

[e]Sources of data according to Grimont and Grimont (1992)

[f]Type strain considered only, no information in literature

antagonist at least towards *F. solani*. Since the *Fusarium* spp. are the causative agent of wilt diseases in a variety of plants of economic interest, it would be interesting to further investigate the capability of *E. ludwigii* BNM 0357 to antagonize the fungus and extend the study to other species of *Fusarium*. BNM 0357 inoculation promoted a slight, statistically significant rise in shoot fresh weight and length, an effect which was not observed at all in plants inoculated with *A. brasilense*. A relatively more significant effect, also restricted to BNM 0357 inoculation, was observed on root fresh weight that increases by 50%. Strengthening of the root system in addition to the capability of the isolate to facilitate solubilization of mineral phosphate could be an important trait for BNM 0357 to improve plant growth depending on the nutritional resources of the soil. In this regard, it is important to emphasize that the Andisol from where BNM 0357 was isolated usually shows high phosphorus sorption capacity (Campillo et al. 2005) making the presence of phosphorus solubilizing microorganisms highly profitable for crops in such soil ecosystem.

8.4.3 *Enterobacter radicincitans* sp.: A Novel Plant Growth Promoting Species

This is based on the report of Kampfer et al. (2005) *E. radicincitans* sp. nov., a PGP species of the family Enterobacteriaceae. According to this study this organism produced acids from various sugars. *E. radicincitans* forms rod-shaped cells 0.8–1.2 mm in length and 1.0–1.6 mm in width, is Gram-negative and motile in nature. Colonies on nutrient agar are beige pigmented, 2–3 mm in diameter and mucoid. The optimum growth temperature is about 30° C, growth does not occur at 10°C and below and at 45°C and above (Kampfer et al. 1991). It is cytochrome oxidase negative and catalase positive. Strain D5/23T showed a positive arginine dihydrolyse reaction and was negative in production of urease, lysine decarboxylase, ornithine decarboxylase but positive to that of Esculin. Tryptophane deaminase, indole production, and H_2S production were negative. The Voges–Proskauer test was positive after 48 h of incubation. Citrate and malonate were positive. Acid was produced from the following compounds: glucose, lactose (after 48 h), sucrose, D-mannitol, dulcitol, salicin, sorbitol, L-arabinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, and D-mannose. No acid production was observed for adonitol, inositol, raffinose, methyl-D-glucoside, erythritol, melibiose, and D-arabitol.

Utilization of a wide range of C and N sources that include *N*-acetylglucosamine, L-arabinose, L-arbutine, D-cellobiose, D-galactose, D-fructose, gluconate, D-glucose, D-maltose, D-mannose, L-rhamnose, D-ribose, sucrose, salicine, D-trehalose, D-xylose, D-maltitol, D-sorbitol, D-mannitol, acetate, cis-aconitate, trans-aconitate, citrate, fumarate, glutarate, DL-lactate, L-malate, pyruvate, L-aspartate, L-alanine, L-proline, and L-serine was positive. The following compounds are not utilized as sole sources

of carbon: *N*-acetylgalactosamine, α-D-melibiose, adonitol, i-inositol, putrescine, propionate, adipate, azelate, 4-aminobutyrate, DL-3 hydroxybutyrate, mesaconate, itaconate, 2-oxoglutarate, suberate, β-alanine, L-ornithine, L-phenylalanine, L-histidine, L-leucine, L-tryptophane, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate. The strain also shows nitrogenase activity, auxine and cytokinine production (Kampfer et al. 2005). This bacterium shows mesophilic, chemoorganotrophic, facultatively anaerobic growth; these biochemicals support substrate utilization profile for *E. radicincitans* with their PGP potentials.

8.4.4 *Enterobacter asburiae* PSI3 *and E. asburiae* PS2: Secreting Organic Acid and Phytotoxic Effect of Cd Metal and Phosphate Solubilization Influenced by Fungicide

Several other bacteria that are known to promote plant growth in the presence of Cd have PGPR characteristics, which are the implicated mechanisms that contribute to the improved growth in presence of Cd stress in their host plants (Safronova et al. 2006; Sinha and Mukherjee 2008). In the present case, the role of organic acid secretion by *E. asburiae* PSI3 in imparting Cd tolerance to mung bean plants was explored by exogenous application of pure organic acids to seedlings cultivated under hydroponics. Under this mode of cultivation (as against in soil), the plantlets were very highly sensitive to Cd and had complete arrest of root elongation at 50 μM $CdCl_2$. This is in agreement with the observation that legume crops are highly sensitive to Cd (Inoune et al. 1994) and also with the report that the extent of the damaging effect of Cd in hydroponics differs from sand culture (Metwally et al. 2005). In soils the reduced toxicity may be due to binding of the metal ion to clay surfaces or to organic matter.

Root elongation rate is an important parameter depicting robustness of the plants, as efficient root development allows exploring for water and mineral nutrients. A decrease in root elongation due to heavy metal has often been reported (Burd et al. 1998; Metwally et al. 2005; Parker and Pedler 1998). When various organic acids were added to plant roots exposed to toxic levels of Cd, there was a partial relief of its effect on root elongation presumably due to the complexation of the metal ions with the acid anion. This was further supported by the reduction in root associated Cd as seen by hematoxylin staining of roots treated with organic acid. Nigam et al. (2000) have argued that metal:organic acid complex is less phytotoxic than the free form of Cd. Parker and Pedler (1998) who described root elongation responses of Al treated *T. aestivum* to exogenously added malate have shown that exogenous addition of organic acid to seedling roots can reduce metal toxicity to plants. Efficacy of different organic acids in the chelation of Cd^{2+}, as determined by hematoxylin competition assay, showed that among the organic

acids tested, citrate and oxalate are most efficient at releasing Cd^{2+} from its hematoxylin complex in compliance with the formation constants (Ryan et al. 2001). Of special interest is the observation that gluconate, a monocarboxylate, is also significantly proficient in binding to Cd^{2+}. Although this acid has never been reported to be secreted by plant roots, it is one of the major organic acids secreted by several rhizosphere bacteria (Goldstein 1995) and may be of significance in microbially mediated alleviation of metal toxicity (Kavita et al. 2008).

Phosphate solubilization potentials of *E. asburiae* strain PS2 was described by Ahmad and Khan (2010). In the presence of varying concentrations of fungicides, P solubilization was checked both qualitatively and quantitatively using a solid and liquid Pikovskaya medium. In general, when the concentration of each fungicide was increased from 1× to 3×, the size of the halo decreased considerably ($p < 0.05$). The effect of three times the recommended rate (3×) of each fungicide was most adverse on the halo formation compared to the recommended (1×) and two times the recommended rate (2×). The order of toxicity of the fungicides at 3× on the halo size (solubilization index) was: tebuconazole > hexaconazole = metalaxyl > kitazin. In addition, the amount of P-solubilized in the liquid medium decreased corresponded with the increasing concentrations of fungicides. Maximum reduction of the P-solubilizing activity of the *E. asburiae* strain PS2 in the broth was found to be 67, 89, and 93% over the control when tebuconazole at 100, 200, and 300 μg/L, respectively, was added to the medium.

8.4.5 *Enterobacter sakazakii* (Cronobacter): Evidence for Plant Association

The isolation of *Cronobacter* organisms has been reported from a wide spectrum of environmental sources including water, soil, dust from households, and food production lines, as well as from foods such as fruits, vegetables, herbs, cereals, and grains. In addition, *Cronobacter* spp. has been isolated from lemon root stocks (Gardner et al. 1982), wheat (Forlani et al. 1995), rice (Yang et al. 1999) and soybean plants (Kuklinsky-Sobral et al. 2004). Some physiological traits exhibited by the organisms showed the ability to produce a yellow pigment (Lehner et al. 2006), the formation of a gum-like extracellular polysaccharide (Lehner et al. 2005) as well as its ability to resist desiccation during long dry periods (Riedel and Lehner 2007) suggest that they might be of environmental origin. However, the natural habitats of *Cronobacter* spp. are still unknown. In a review article by Berg et al. (2005), the rhizosphere was described as a reservoir for several opportunistic human pathogens including species closely related to *Cronobacter* spp., such as *Enterobacter cloacae* (Berg et al. 2005). It was proposed that mechanisms involved in the interaction between plant-associated bacteria and their host plants are similar

to those responsible for pathogenicity in bacteria and that these mechanism may also be involved in colonizing the human body (Rahme et al. 1995; Cao et al. 2001). Schmid et al. (2009) stated that members of the genus *Cronobacter* can be readily isolated from plant roots and that clinical as well as plant isolates are capable of developing epiphytic and endophytic colonization of tomato and maize roots. It is interesting to note that *Cronobacter* spp. can produce factors potentially beneficial to plant growth. This provides evidence for plants as the original natural habitat of *Cronobacter* spp. Other questions such as the mode of entry, potential proliferation of bacteria within plant cells, and/or whether the cells are transported by an (active/passive) mechanism to other parts of the plants (fruits, seeds) will be subject of further investigation.

8.4.6 *Enterobacter cancerogenus*: A Novel Plant Growth Promoting Agent

Enterobacter cancerogenus (can. cer. o'ge. nus. L. n. *cancer*, crab, the disease cancer; L. v. *gigno*, to produce; L. masc. adj. *cancerogenus*, cancer inducing). The bacteria isolated by UroSeviC from poplars (*Populus* species) affected by a canker disease were described and designated *Erwinia cancerogena* in 1966 (UroSevie 1966). Although Lelliott (1974) and Lelliott and Dickey (1984) have indicated that *Erwinia cancerogena* probably is a species of *Enterobacter* because it produces positive reactions for arginine and ornithine decarboxylase, additional data have not been reported to substantiate the suggested change to *Enterobacter*. *Enterobacter cancerogenus* is Gram-negative, straight rods that are motile with peritrichous flagella and are facultatively anaerobic. This species could give positive results for following characteristics: catalase production, nitrate reduction, Voges–Proskauer reaction, KCN tolerance, esculin hydrolysis, β-galactosidase production, utilization of acetate, citrate, glutamate, DL-lactate, malate, succinate, L-alanine, DL-α-alanine, and L-serine, production of acid from L-arabinose, D-xylose, D-ribose, D-glucose, D-lactose, D-galactose, L-rhamnose, D-mannose, D-fructose, D-trehalose, D-cellobiose, D-mannitol, glycerol, salicin, mucate, pyruvate, and α-D-galacturonate. Liquefaction of gelatin at 27°C is evident at 15 days, but exhibits negative for the following characteristics: pigment production, oxidase, the methyl red test, production of deoxyribonuclease, hydrogen sulfide, lipase, lysine decarboxylase, and urease, phenylalanine deamination, reducing substances produced from sucrose, utilization of alginate, benzoate, propionate, and sodium potassium tartrate; production of acid from L-sorbose, melezitose, ethanol, adonitol, i-erythritol, inulin, glycogen, chitin, and D-tartaric acid, production of gas from D-arabinose and myo-inositol (Dickey and Zumoff 1988). *Enterobacter cancerogenes* MSA2 supplemented with 1% carboxymethylcellulose showed overall plant (*Jatropha curcas* L.) growth promotion effect resulting in enhanced root length, fresh root mass, fresh shoot mass, dry root

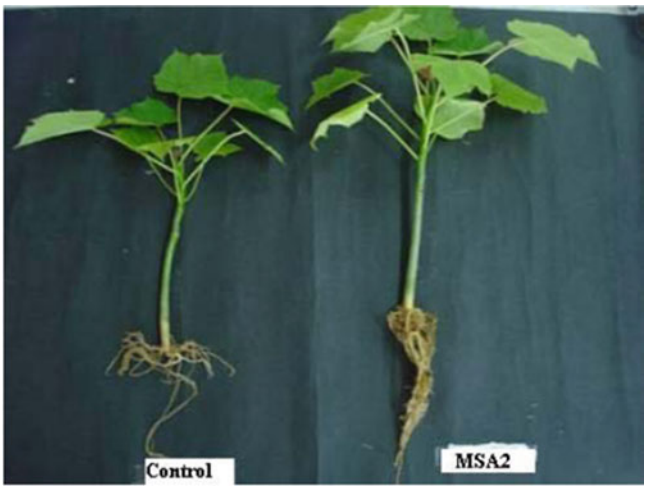

Fig. 8.2 Effect of *Enterobacter cancerogenus* MSA2 on growth of *Jatropha curcas* after 90 DAS in comparison to control

mass, dry shoot mass, number of leaf, chlorophyll content, and biomass over control under the days of experimental observation (Fig. 8.2) (Jha 2011).

8.5 Conclusion

Colonization of the rhizosphere by micro-organisms results in modifications in plant growth and development. This chapter examines the mechanisms involved in growth promotion by plant growth-promoting rhizobacteria of *Enterobacter* species, a common soil bacterium that enhances development of plant root systems and protects host plants against pathogen infection. Continued research with colonization and biofilm formation by these bacterial genera also holds potential for developing biofertilizer and biocontrol agents that may be self perpetuating within the colonizing host plants. Focusing research in these areas may also be aimed to establish *Enterobacter* spp. as promising/potential PGPR. Microbes being an integral component of any soil ecosystem provide life to the soil. Native soils minus microbes are merely dead material. It is now widely being recognized that the presence and abundance of microbial wealth provide soils richness in terms of making available slow release of mineral nutrients, continuous breaking down of complex macro-molecules and natural products into simpler ones to enrich beneficial substances, maintaining physicochemical properties of the soils and most essentially, providing support to the plants in terms of growth enhancement and protection against diseases and pests through their metabolic activities that go on in the soil along day and night.

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References

Ahmad M, Khan MS (2010) Plant growth promoting activities of phosphate solubilizing *Enterobacter asburiae* as influenced by Fungicides. Eur Asia J Biosci 4:88–95

Andrews AE, Lawley B, Pittard AJ (1991) Mutational analysis of repression and activation of the *tyrP* gene in *Escherichia coli*. J Bacteriol 173:5068–5078

Bangera MG, Thomashaw LS (1996) Characterization of a genomic locus required for synthesis of the antibiotic 2,4-diacetylphloroglucinol by the biological control agent *Pseudomonas fluorescens* Q2-87. Mol Plant Microb Interact 9:83–90

Bent E, Tuzun S, Chanway CP, Enebak S (2001) Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. Can J Microbiol 47:793–800

Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Appl Environ Microbiol 68:3328–3338

Berg G, Eberl L, Hartmann A (2005) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environ Microbiol 7:1673–1685

Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V (1997) The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1462

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Boddey RM, Da Silva LG, Reis V, Alves BJR, Urquiaga S (2000) Assessment of bacterial nitrogen fixation in grass species. In: Triplett EW (ed) Prokaryotic nitrogen fixation: a model system for analysis of a biological process. Horizon Scientific, Wymondham, UK, pp 705–726

Brandl MT, Lindow SE (1996) Cloning and characterization of a locus encoding an indolepyruvate decarboxylase involved in indole-3-acetic acid synthesis in *Erwinia herbicola*. Appl Environ Microbiol 62:4121–4128

Brenner DJ, Farmer J Jr (2005) In: Garrity GM (ed) Bergey's manual of systematic bacteriology, vol 2. Springer, Berlin, Heidelberg, New York, pp 587–850

Briely IJ, Thornton I (1983) Primary observation on the effect on heavy metals from mining and smelting on nitrogen-fixing bacteria in some British soils. Miner Environ 1:112–119

Burd GI, Dixan DG, Glick BR (1998) A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Campbell R, Greaves MP (1990) Anatomy and community structure of the rhizosphere. In: Lynch JM (ed) The rhizosphere. Wiley, Chichester, England, pp 11–34

Campillo R, Urquiaga S, Undurraga P, Pino I, Boddey RM (2005) Strategies to optimise biological nitrogen fixation in legume/grass pastures in the southern region of Chile. Plant Soil 273:57–67

Cao H, Baldini RL, Rahme LG (2001) Common mechanisms for pathogens of plants and animals. Annu Rev Phytopathol 39:259–284

Carlot M, Giacomini A, Casella S (2002) Aspects of plant-microbe interactions in heavy metal polluted soil. Acta Biotechnol 22:13–20

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Cervantes C, Gutierrez-Corana F (1994) Copper resistance mechanism in bacteria and fungi. FEMS Microbiol Rev 14:121–137

Chanway CP, Holl FB (1993) First year yield performance of spruce seedlings inoculated with plant growth promoting rhizobacteria. Can J Microbiol 39:1084–1088

Chincholkar SB, Chaudhari BL, Rane MR, Sarode PD (2007) Fungal phytopathogen suppression using siderophoregenic bio-inoculants. In: Mukerji KG, Chincholkar SB (eds) Biological control of plant diseases: current concepts. Haworth Press, USA, pp 401–417

Chung YR, Brenner DJ, Steigerwalt AG, Kim BS, Kim HT, Cho KY (1993) *Enterobacter pyrinus* sp. nov. an organism associated with brown leaf spot disease of pear trees. Int J Syst Bacteriol 43:157–161

Conrad R (1996) Soil micro-organisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiol Rev 60:609–643

Constantinescu F (2001) Extraction and identification of antifungal metabolites produced by some *B. subtilis* strains. Analele Institutului de Cercetari Pentru Cereale Protectia Plantelor 31:17–23

Costacurta A, Keijers V, Vanderleyden J (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. Mol Gen Genet 243:463–472

De Meyer G, Capieau K, Audenaert K, Buchala A, Metraux JP, Hofte M (1999) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. Mol Plant Microb Interact 12:450–458

Deepa CK, Dastager SG, Pandey A (2010) Isolation and characterization of plant growth promoting bacteria from non rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. World J Microbiol Biotechnol 26:1233–1240

Dickey RS, Zumoff CH (1988) Emended description of *Enterobacter cancerogenus comb.* nov. (formerly *Erwinia cancerogena*). Int J Syst Bacteriol 38:371–374

English MM, Coulson TJD, Horsman SR, Patten CL (2010) Overexpression of hns in the plant growth-promoting bacterium *Enterobacter cloacae* UW5 increases root colonization. J Appl Microbiol 108:2180–2190

Farmer J Jr (2005) In: Garrity GM (ed) Bergey's manual of systematic bacteriology, vol 2B. Springer, New York, pp 694–698

Forlani G, Mantelli M, Branzoni M, Nielsen F, Favilli F (1995) Differential sensitivity of plant-associated bacteria to sulfonylurea and imidazolinone herbicides. Plant Soil 176:243–253

Fravel DR (1988) Role of antibiosis in the biocontrol of plant diseases. Annu Rev Phytopathol 26:75–91

Gardner JM, Feldman AW, Zablotowicz RM (1982) Identity and behavior of xylem-residing bacteria in rough lemon roots of Florida citrus trees. Appl Environ Microbiol 43:1335–1342

Garrity GM (2005) Bergey's manual of systematic bacteriology, vol 2. Springer, Berlin, Heidelberg, New York, pp 1–1085

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol Agric Hortic 12:185–193

Grimont F, Grimont PA (1992) The genus *Enterobacter*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The procaryotes. Springer, New York, pp 2797–2815

Gyaneshwar P, Parekh LJ, Archana G, Poole PS, Collins MD, Hustson RA (1999) Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by *Enterobacter asburiae PSI3*. FEMS Microbiol Lett 171:223–229

Hasnain S, Sabri AN (1996) Growth stimulation of *Triticum aestivum* seedlings under Cr-stresses by non rhizospheric pseudomonad strains. In: Abstracts of the 7th international symposium on biological nitrogen fixation with non-legumes, p 36

Hazel JR, Williams EE (1990) The role of alteration in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog Lipid Res 29:167–227

Hoffmann H, Roggenkamp A (2003) Population genetics of the nomenspecies *Enterobacter cloacae*. Appl Environ Microbiol 69:5306–5318

Hoffmanna H, Stindlb S, Stumpf A, Mehlen A, Monget D, Heesemann J, Schleifer KH, Roggenkamp A (2005) Description of *Enterobacter ludwigii* sp. nov., a novel *Enterobacter* species of clinical relevance. Syst Appl Microbiol 28:206–212

Hommais F, Krin E, Laurent-Winter C, Soutourina O, Malpertuy A, Le Caer JP, Danchin A, Bertin P (2001) Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. Mol Microbiol 40:20–36

Honma M, Shimomura T (1978) Metabolism of l-aminocyclopropane-1-carboxylic acid. Agric Biol Chem 42:825–1831

Hormaeche E, Edwards PR (1960) A proposed genus *Enterobacter*. Int Bull Bacteriol Nomencl Taxon 10:71–74

Inoue K, Sugiyama K, Kosako Y, Sakazaki R, Yamai S (2000) *Enterobacter cowanii* sp. nov., a new species of the family Enterobacteriaceae. Curr Microbiol 41:417–420

Inoune M, Ninomiya S, Tohoyama H, Joho M, Murayama T (1994) Different characteristics of roots in the cadmium tolerance and Cd binding complex formation between mono- and dicotyledenous plants. J Plant Res 107:201–207

Jackson MB (1997) Hormones from roots as signal for the shoots of stressed plants. Trends Plant Sci 2:22–28

James EK (2000) Nitrogen fixation in endophytic and associative symbiosis. Field Crop Res 65:197–209

James EK, Olivares FL, Baldani JI, Döbereiner J (1997) *Herbaspirillum,* an endophytic diazotroph colonizing vascular tissue in leaves of *Sorghum bicolor* L. Moench. J Exp Bot 48:785–797

Jha CK (2011) Isolation Characterization and development of consortia of PGPR for the growth of *Jatropha curcas*. Ph.D thesis, Gujarat University.

Jia YJ, Ito H, Matsui H, Honma M (2006) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase induced by ACC synthesized and accumulated in *Penicillium citrinum* intracellular spaces. Biosci Biotechnol Biochem 64:299–305

Kampfer P, Steiof M, Dott W (1991) Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. Microb Ecol 21:227–251

Kampfer P, Ruppelb S, Remus R (2005) *Enterobacter radicincitans* sp. nov., a plant growth promoting species of the family Enterobacteriaceae. Syst Appl Microbiol 28:213–221

Kavita B, Shukla S, Naresh Kumar G, Archana G (2008) Amelioration of phytotoxic effects of Cd on mung bean seedlings by gluconic acid secreting rhizobacterium *Enterobacter asburiae* PSI3 and implication of role of organic acid. World J Microbiol Biotechnol 24:2965–2972

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Lankford CE (1973) Bacterial assimilation of iron. Crit Rev Microbiol 2:273–331

Lehner A, Riedel K, Eberl L, Breeuwer P, Diep B, Stephan R (2005) Biofilm formation, EPS production and cell-to-cell signalling in various *Enterobacter sakazakii* strains: aspects promoting environmental persistence. J Food Prot 68:2287–2294

Lehner A, Grimm M, Rattei T, Ruepp A, Frishman D, Manzardo GGG, Stephan R (2006) Cloning and characterization of *Enterobacter sakazakii* pigment genes and in situ spectroscopic analysis of the pigment. FEMS Microbiol Lett 265:244–248

Lelliott RA (1974) Genus XII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumweide, Rogers and Smith 1920. In: Buchanan RE, Gibbons NE (eds) Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore, pp 332–339

Lelliott RA, Dickey RS (1984) Genus VII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams and Wilkins, Baltimore, pp 469–476

Lucchini S, Rowley G, Goldberg MD, Hurd D, Harrison M, Hinton JC (2006) H-NS mediates the silencing of laterally acquired genes in bacteria. PLoS Pathog 2:81

Malik KA, Bilal R, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing, plant growth-promoting rhizobacteria (PGPR) with kallar grass and rice. Plant Soil 194:37–44

Martinez-Granero F, Rivilla R, Martin M (2006) Rhizosphere selection of highly motile phenotypic variants of *Pseudomonas fluorescens* with enhanced competitive colonization ability. Appl Environ Microbiol 72:3429–3434

Metwally A, Safronova V, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot 56:167–178

Navarre WW, Porwollik S, Wang Y, McClelland M, Rosen H, Libby SJ, Fang FC (2006) Selective silencing of foreign DNA with low GC content by the H-NS protein in *Salmonella*. Science 313:236–238

Nigam R, Srivastva S, Prakash S, Srivastava MM (2000) Effect of organic acids on availability of cadmium in wheat. Chem Spec Bioavailab 12:125–132

O'Hara CM, Steigerwalt AG, Hill BC, Farmer JJ 3rd, Fanning GR, Brenner DJ (1989) *Enterobacter hormaechei* a new species of the family Enterobacteriaceae formerly known as enteric group 75. J Clin Microbiol 27:2046–2049

Ohsumi Y, Katsuhiko K, Yasuhiro A (1988) Changes induced in the permeability barrier of the yeast plasma membrane by cupric ion. J Bacteriol 170:2676–2682

Ono S, Goldberg MD, Olsson T, Esposito D, Hinton JC, Ladbury JE (2005) H-NS is a part of a thermally controlled mechanism for bacterial gene regulation. Biochem J 391:203–213

Paradis S, Boissinot M, Paquette N, Bélanger SD, Martel EA, Boudreau DK, Picard FJ, Ouellette M, Roy PH, Bergeron MG (2005) Phylogeny of the *Enterobacteriaceae* based on genes encoding elongation factor Tu and F-ATPase β-subunit. Int J Syst Evol Microbiol 55:2013–2025

Parker DR, Pedler JF (1998) Probing the malate hypothesis of differential aluminium tolerance in wheat by using other rhizotoxic ions as proxies for Al. Planta 205:389–396

Pavan ME, Franco RJ, Rodriguez JM, Gadaleta P, Abbott SL, Janda JM, Zorzópulos J (2005) Phylogenetic relationships of the genus *Kluyvera*: transfer of *Enterobacter intermedius* Izard *et al*. 1980 to the genus *Kluyvera* as *Kluyvera intermedia* comb. nov. and reclassification of *Kluyvera cochleae* as a later synonym of *K. intermedia*. Int J Syst Evol Microbiol 55:437–442

Persello-Cartieaux F (2003) Tales from the underground: molecular plant–rhizobia interactions. Plant Cell Environ 26:189–199

Pittard J, Camakaris H, Yang J (2005) The TyrR regulon. Mol Microbiol 55:16–26

Rahme LG, Stevens EJ, Wolfort SF, Shoa J, Thomkins RG, Ausubel FM (1995) Common virulence factors for bacterial pathogenicity in plants and animals. Science 268:1899–1902

Riedel K, Lehner A (2007) Identification of proteins involved in osmotic stress response in *Enterobacter sakazakii* by proteomics. Proteomics 7:1217–1231

Rodríguez-Díaz M, Belén RG, Clementina PC, Maria Victoria M, Jesús G (2008) A review on the taxonomy and possible screening traits of plant growth promoting rhizobacteria. In: Iqbal A, John P, Shamsul H (eds) Plant–bacteria interactions. strategies and techniques to promote plant growth. WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim

Romero D, de Vicente A, Rakotoaly RH, Dufour SE, Veening JW, Arrebola E, Cazorla FM, Kuipers OP, Paquot M, Perez-García A (2007) The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. Mol Plant Microb Interact 20:430–440

Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:527–556

Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3 pyruvate decarboxylase is regulated by TyrR in *Enterobacter cloacae* UW5. J Bacteriol 190:7200–7208

Ryu C-M, Farag MA, Hu C-H, Reddy MS, Wie H-X (2003) Bacterial volatiles promote growth of *Arabidopsis*. Proc Natl Acad Sci USA 100:4927–4932

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol 134:1017–1026

Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Saraf M, Jha CK, Patel D (2010) The role of ACC deaminase producing PGPR in sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 365–385

Saravanakumar D, Samiyappan R (2007) ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. J Appl Microbiol 102:1283–1292

Schmid M, Iversen C, Gontia I, Stephan R, Hofmann A, Hartmann A, Jha B, Eberl L, Riedel K, Lehner A (2009) Evidence for a plant-associated natural habitat for *Cronobacter* spp. Res Microbiol 160:608–614

Shah S, Li J, Moffatt BA, Glick BR (1998) Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria. Can J Microbiol 44:833–843

Shoebitz M, Claudia MR, Martín AP, María LC, Luigi C, José AC (2009) Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from Lolium perenne rhizosphere. Soil Biol Biochem 41:1768–1774

Siebert M, Sommer S, Li SM, Wang ZX, Severin K (1996) Genetic engineering of plant secondary metabolism. Accumulation of 4-hydroxybenzoate glucosides as a result of the expression of the bacterial ubiC gene in tobacco. Plant Physiol 112:811–819

Singh N, Pandey P, Dubey RC, Maheshwari DK (2008) Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* by rhizosphere competent *Bacillus subtilis* BN1. World J Microbiol Biotechnol 24:1669–1679

Singh N, Kumar S, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Biocontrol of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promontory activity in chir pine. Crop Prot 29:1142–1147

Sinha S, Mukherjee SK (2008) Cadmium-induced siderophore production by a high Cd-resistant bacterial strain relieved Cd toxicity in plants through root colonization. Curr Microbiol 56:55–60

Stella S, Falconi M, Lammi M, Gualerzi CO, Pon CL (2006) Environmental control of the in vivo oligomerization of nucleoid protein H-NS. J Mol Biol 355:169–174

Taghavi S, Lelie D, Hoffman A, Zhang YB, Walla MD, Vangronsveld J, Newman L, Monchy S (2010) Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. PLoS Genet 6:1–15

Triplett E (1996) Diazotrophic endophytes: progress and prospects for nitrogen fixation in monocots. Plant Soil 186:29–38

Turnbull GA, Morgan JA, Whipps JM, Saunders JR (2001) The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment and colonisation of wheat roots. FEMS Microbiol Ecol 36:21–31

UroSevie B (1966) Rakovina topolu piisobenFi-baktkrii *Erwinia cuncerogena* n. sp. Lesnicky Casopis 12:493–505

Vansuy G, Robin A, Briat JF, Curie C, Lemanceau P (2007) Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. Mol Plant Microb Interact 20:441–447

Voisard C, Keel C, Haas D, Défago G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351–358

Wang KL, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14:131–151

Weigel C, Schmidt A, Ruckert B, Lurz R, Messer W (1997) DnaA protein binding to individual DnaA boxes in the *Escherichia coli* replication origin, oriC. EMBO J 16:6574–6583

Windstam S, Nelson EB (2008) Differential interference with *Pythium ultimum* sporangial activation and germination by *Enterobacter cloacae* in the corn and cucumber spermospheres. Appl Environ Microbiol 74:4285–4291

Yang HL, Sun XL, Song W, Wang YS, Cai MY (1999) Screening, identification and distribution of endophytic associative diazotrophs isolated from rice plants. Acta Bot Sin 41:927–931

Zhang H, Xie X, Kim M-S, Kornyeyev DA, Holaday S, Paré PW (2008) Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels *in planta*. Plant J 56:264–273

Zhender GW, Yao C, Murphy JF, Sikora ER, Kloepper JW, Schuster DJ, Polston JE (1999) Microbe induced resistance against pathogens and herbivores: evidence of effectiveness in agriculture. In: Agarwal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores: biochemistry, ecology and agriculture. APS, St. Paul, MN, p 33