Chapter-1

INTRODUCTION
1. INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are a group of naturally occurring beneficial soil bacteria, mainly present in the rhizosphere at root surfaces and have the capability to stimulate plant growth. The term PGPR was first used by Kloeper and Schroth (1978). Rhizosphere is the defined as the region immediately outside the root and a dynamic zone for microbial activities and is of crucial importance for plant health and nutrition. It has a high level of microbial activity, particularly because of nutrients secreted by plant roots in the form of soluble exudates such as amino acids, organic acids and other photosynthates (Nihorimbere et al., 2011). PGPR can affect plant growth by a wide range of mechanisms such as solubilization of inorganic phosphate, production of phytohormones, siderophore and organic acids, lowering of plant ethylene levels, nitrogen fixation and biocontrol of plant diseases (Muleta et al., 2007; Rani et al., 2012). Along with the promotion of plant growth, PGPR also help in sustainable agricultural development, thereby protecting the environment and thus help in maintaining the ecological balance. Bacterial plant growth promotion is a well established and complex phenomenon, and is often achieved by the activities of multiple plant growth promoting traits exhibited by the associated bacteria (Lifshitz et al., 1987). The inoculation of cultivated plants with PGPR is a promising agricultural approach as it reduces the rate of fertilizer, fungicide and pesticide use in cultivated crops. Therefore, the use of such beneficial bacteria as biofertilizers and biocontrol agents has attracted increased interest worldwide in attempts to achieve sustainability in agriculture (Datta et al., 2011). Several PGPR strains including Alcaligenes, Azotobacter, Acetobacter, Azospirillum, Bacillus, Brevidomonas, Pseudomonas, Provedinecia and Burkholderia have been identified and evaluated for the plant growth promotion (Kloeper et al., 1989; Selvakumar et al., 2008; Grover et al., 2009; Nain et al., 2010 and Rana et al., 2011).

Medicinal plants constitute a large segment of the flora, which provide raw materials for pharmaceutical, cosmetic and fragrance industries (Karthikeyan et al., 2008). Medicinal plants support a great diversity of microflora in their rhizosphere including PGPR (Malleswari and Bagyanarayana, 2013). The clear understanding and
management of microbial species associated with them known to improve their yield and quality (Jaleel et al., 2007). The demand for medicinal plants is increasing worldwide due to the growing recognition of biological products, being non-toxic, having no side effects and affordable (Sekar and Kandavel, 2010). One such unique medicinal plant which is found to be a boon for diabetic patients is *Stevia* whose botanical name is *Stevia rebaudiana*. It belongs to Asteraceae family and is native to certain regions of South America such as Paraguay and Brazil (Sivaram and Mukundan, 2003). The leaves of *Stevia* are the source of diterpene glycosides such as stevioside (ST) and rebaudioside (Ahmed and Salahin, 2007). Stevioside, the main sweet component in the leaves of *S. rebaudiana*, tastes about 300 times sweeter than sucrose (Geuns, 2003). Thus, it is considered to be a sugar substitute and commercial sweetener both in the form of stevioside and extract. Beside ST, *Stevia* also contains significant quantities of chlorogenic acid, which has hypoglycemic effects (Khramov and Dmitrienko, 2000; Gregersen et al., 2004). Some ingredients of *Stevia* are commercially used as a low-caloric sweetener, i.e., as a sugar substitute. Apart from this, *Stevia* is rich in various nutrients such as Magnesium, Miocene, riboflavin, Zinc, Chromium, Selenium, Calcium and Phosphorus and also containing substantial amount of Protein (Chatsudthipong and Muanprasat, 2009). This implies that uses of *Stevia* leaves to control various diseases would see dramatic increase in near future.

The amount of active principles depends on total biomass, which further depends on climatic features, agro-techniques, water management and fertilizer applications. Hence, there is a need to enhance biomass through cultural techniques, application of manures, and fertilizers, including biofertilizers. It has been demonstrated that chemical fertilizer has increased crop yield, but has also caused deleterious effects such as soil acidification and production of the greenhouse gas nitrous oxide (N$_2$O) through denitrification on ecosystems (Biswas et al., 2000). Therefore, one potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with biofertilizers such as PGPR (Adesemoye et al., 2009).

Biofertilizers are the products containing living cells of different types of microorganisms that enrich the nutrient quality of soil and supply the plant with
nutrients (Vessey, 2003). Most biofertilizers belong to one of the following categories: nitrogen fixing, phosphate solubilizing and mobilizing, and plant growth promoting rhizobacteria. Biological nitrogen fixation by rhizospheric bacteria is an important property contributing to plant growth (Gothwal et al., 2008). For example, symbiotic root-nodulating rhizobia are the most prominent among the nitrogen-fixing microorganisms. Phosphorus (P) is one of major nutrient and world’s second largest agricultural chemical required by plant for growth and development. Several rhizobacteria are found to solubilize plant-available phosphate from either organic or inorganic bound phosphate and thereby make it available to the plants and thus act as biofertilizers (Lugtenberg et al., 2009). Phosphate solubilizing bacteria convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid, thereby improves the overall health of plants (Chung et al., 2005; Gulati et al., 2010).

Plant growth hormones produced by PGPR are signal molecules acting as chemical messengers and play a fundamental role in the growth and development of the plants (Martinez-Viveros et al., 2010). The phytohormone auxin produced by fluorescent Pseudomonads is one of the best understood examples (Kamilova et al., 2006; Khare and Arora, 2010). Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism produced by several microorganisms including PGPR (Elliot and Lynch, 1985). IAA is released as a secondary metabolite because of the rich supplies of substrates exuded from the roots (Ahmad et al., 2005).

PGPR were also reported to suppress the plant pathogens, of which Pseudomonas sp. and Bacillus sp. are important as these are aggressive colonizers of the rhizosphere of various crops and have broad spectrum of antagonistic activity against many pathogens (Weller et al., 2002). Biocontrol bacterial species generally employ an array of mechanisms such as antibiosis, competition, production of hydrocyanic acid (HCN), siderophore and antifungal compounds to antagonize pathogens (Singh et al., 2010). Various rhizobacteria, including for example Burkholderia cepacia, Staphylococcus epidermidis, and strains of the Bacillus subtilis group, stimulate plant growth by the emission of volatile organic compounds such as
hydrogen cyanide and ammonia (Ryu et al., 2003; Kai et al., 2009; Effmert et al., 2012; Bitas et al., 2013). Voisard et al. (1989) reported that HCN production by the rhizobacteria plays a significant role in the biological control of pathogens by acting as an inducer of plant resistance. It was also reported that HCN indirectly influences plant growth promotion (Kumar et al., 2012). Afsharmanesh et al. (2010) suggested that fungal growth is mainly inhibited by HCN production and siderophore production. This in turn can indirectly enhance the plant growth by keeping plant healthy and disease free (Glick and Pasternak, 2003). Additionally, secretion of siderophore and production of chitinases, glucanases, cellulases, lipases and other lytic enzymes by PGPR can affect plant growth as these are also found to suppress the growth of fungal pathogens (Kloepper et al., 1980). Siderophores are high affinity iron chelating compounds produced by microorganisms that increase the availability and uptake of iron, thereby helps in directly supplying iron to the plants (Glick et al., 1999). Siderophores also act as biocontrol agent indirectly by depriving iron for fungal pathogens (Ahmad et al., 2008).

In agriculture, phytopathogenic fungi cause plant diseases and much loss of crop yields. Like other cultivated plants, medicinal and aromatic plants are also attacked by a number of fungi (Paul and Singh, 2002). The disease protection measures of medicinal plants are still restricted to the application of various chemical fungicides which strictly do not fit with the basic theory of usefulness of herbal drugs. Also, the residual effects of different chemicals eventually contaminate the purity of such plant drugs and are also of serious concern from environmental point of view (Sharma et al., 2004). Therefore, biological control agents are gaining importance in the field of disease management of medicinal plants (Mathivanan et al., 2005). Many researchers have reported that effective colonization by PGPR contributed to the successful suppression of plant pathogens and thus these can be considered as potential use of biocontrol agents as replacements for agrochemicals (Cavaglieri et al., 2005; Demoz and Korsten 2006; Jayraj et al., 2007; Guo et al., 2007). In recent years, certain plant associated rhizobacteria belonging to various genera like Pseudomonas, Burkholderia and Bacillus have drawn attention because of their
potential to suppress the soil borne pathogens causing diseases in different crops (Kazempour, 2004; Cazorla et al., 2007).

Beneficial PGPR increases the drought tolerance and water use efficiency of plants growing in arid regions (Marulanda et al., 2007). The bacteria like *P. fluorescens* can survive under dry conditions and hyperosmolarity. PGPR induces physical and chemical changes in plants that result in enhanced tolerance to abiotic stress (Yang et al., 2009). Ethylene level in the plants can be used as the indicator of plant performance under different conditions. The synthesis of ethylene in plants is directly related to the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) (Li et al., 2000). Under stress the amount of ethylene produced by the plants increases (Glick et al., 1998; Jalili et al., 2009). Some specific microorganisms including PGPR produce an enzyme called ACC deaminase, hydrolyzing ACC into ammonia and α-ketobutyrate (Glick et al., 1998). Hence, applications of rhizobacteria capable of producing ACC deaminase may act as a sink for ACC, which will be very beneficial to the plant growth resulting in enhanced yield under different stressed conditions.

Habitat loss and deforestation coupled with over harvesting has resulted in dwindling population of important medicinal plants around the world. So, direct extraction of natural products from wild medicinal plants to satisfy the current requirement is fast becoming an unrealistic goal. Domestic cultivation of medicinal plants was thought as a viable alternative. But, certain drawbacks including variability in yield and difference in phytochemical profile over wild ones are making it as a last resort (Malairajan et al., 2006). Extensive use of chemicals to control plant diseases has disturbed the delicate ecological balance of the soil, leading to groundwater contamination, development of resistant races of pathogen and health risks to humans. The recognition of PGPR as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (Dey et al., 2004; Herman et al., 2008; Minorsky, 2008). Increased growth and yields of potato, sugar beet, radish and sweet potato (Kloepper and Schrotth, 1978; Farzana et al., 2009; Calvo et al., 2010) have
been reported. Hence, the use of microbial inoculants is a promising agricultural approach that plays a vital role in growth promotion, crop protection through biological disease control and sustained soil fertility (Dilantha et al., 2006).

Keeping in view the major role of PGPR in plant growth promotion and development, the present investigation was undertaken to isolate and characterize native bacteria possessing multiple plant growth promoting properties from the rhizosphere of Stevia plants. To achieve these goals, the present investigation was carried out with following objectives:

1. Isolation of plant growth promoting rhizobacteria from Stevia rhizosphere.
2. Screening of bacterial isolates for desirable properties.
3. Biochemical and Molecular characterization of bacterial isolates.
4. Optimization of cultural conditions for suitable properties.