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INTRODUCTION

Stevia rebaudiana is a perennial herb, commonly referred as to honey leaf, candy leaf and sweet leaf. It belongs to the Asteraceae family and grows up to a height of 1m (Hoekstra and Schaneberg, 2007). Stevia is a common ingredient in beverages and is used as a vasodilator, cardiotonic, anesthetic and anti-inflammatory (Jayaraman *et al*., 2008). It is the world's only natural sweetener with zero calories and zero glycemic index. These attributes makes Stevia a good alternative to sugar or chemical sweeteners (Amzad-Hossain, 2010).

The plant as well as its extract has been used since a long time as a sweetener in various regions viz. South America, Asia, Japan and China. The leaves are found to contain a complex mixture of eight sweet diterpene glycosides, including stevioside, steviolbioside, rebaudiosides (A,B,C,D,E) and dulcoside A (Kim *et al.,* 2002).

Sweet tasting stevioside (STS), a diterpene glycoside present in the leaves of the Stevia plant is 300 times sweeter than sucrose and is non-caloric, non-fermentable, non-discoloring with a long shelf life. Different plant organs, however, contain different amounts of steviol glycosides (SGs) which decline in the order as leaves, flowers, stems, seeds and roots. Along with these glycosides, the plant also contains phenylpropanoids, caffeic acid, scopoletin, umbelliferone, quercetin, avicularian, polystachoside and isoquercitrin extracted from leaves (Uddin *et al*., 2006). The chlorogenic acid has hypoglycemic effect (Gregersen *et al*., 2004). Steviosides have been used as a dietary supplement by the United States since 1995 (Chatsubthibong and Muanprasad, 2009).

In India, the Stevia plant has been introduced in the states of Maharastra, Rajasthan and West Bengal. Like other cultivated plants, medicinal and aromatic plants are also attacked by a number of fungi, bacteria, viruses and nematodes leading to significant quantitative and qualitative losses. Most of the diseases are of fungal origin (Paul and Singh, 2002). Survey for the last five consecutive years confers that *Alternaria* leaf disease was very common in medicinal plants cultivated in various districts of West Bengal, India (Maiti *et al.,* 2007a). A root rot disease caused by *Sclerotium rolfsii* was first reported in major Stevia growing areas of Tamil Nadu (Kamalakannan *et al*., 2007).

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. The residual effects of different chemicals eventually contaminate the purity of such plant drugs and are also of serious concern from the 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).

Biological control is found to be long-lasting, eco-friendly and economical and can be an alternative method to chemical control (Ranjani and Parakhia, 2009). Biocontrol using microbial agents especially plant growth promoting rhizobacteria (PGPR) has been particularly effective under field conditions. PGPR stimulates plant growth, maintains soil health and induces plant defense system against diseases (Nair and Anith, 2009). The use of PGPR has become a common practice in many regions of the world.

The diversity of naturally occurring microorganisms of the rhizosphere and phyllosphere and their potential for biological control of plant pathogens have been examined extensively (Jayraj *et al.,* 2007). Pseudomonads are considered to be important rhizosphere organisms, wherein considerable research is underway globally to exploit the potential. Fluorescent pseudomonads help in maintenance of soil health, protect crops from pathogens and are metabolically and functionally more diverse (Choudhury *et al.,* 2009).

Pseudomonas species are the most diverse and versatile group of microflora of almost all the horticulture and forestry crops and have potential to synthesize different metabolites with diverse biological activities. The ability of soil microorganism to synthesize various metabolites is important in controlling the diseases of plants (Sharma and Kaur, 2010).

Fluorescent pseudomonads are ubiquitous bacteria that are common inhabitants of the plant rhizosphere. Strains of *Pseudomonas* have shown their abilities to suppress a wide variety of fungal root pathogens in many different agricultural crops around the world (Tayung *et al*., 2007).

Fluorescent pseudomonads help in the maintenance of soil health, protect crops from pathogens and are metabolically and functionally diverse (Choudhary *et al*., 2009). Many fluorescent pseudomonads protect plants from soil-borne diseases by the production of antimicrobial secondary metabolites such as 2,4 - diacetyl phloroglucinol (Rezzonico *et al*., 2007).

Pseudomonas can synthesize enzymes that can modulate plant hormone levels, limit the available iron through siderophore production and can also kill the pathogens with antibiotics. In addition, induced systemic resistance by *Pseudomonas* is also thought to be a biocontrol mechanism against plant pathogens (Akhtar and Siddiqui, 2008). Many defense enzymes are involved in defense reaction against plant pathogens. This includes oxidative enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and other phenols that contribute to the formation of defense barriers for reinforcing cell structure (Lavania *et al*., 2006). *Pseudomonas fluorescences* is plant growth-promoting rhizobacteria (PGPR). Most *Pseudomonas fluorescences* isolated from plant rhizosphere can play an important role in the biological control of plant diseases and stimulation of plant growth. Therefore, it is a potential for *Pseudomonas fluorescences* to suppress plant diseases.

For a long time, Gram-negative bacteria, especially *Pseudomonas* strains, have been intensively investigated as biological control agents. However, recently the attention has switched to the Gram-positive members of the aerobic, spore-forming genus *Bacillus*. Among them, *B. subtilis* - a Gram-positive model organism and a prevalent soil inhabitant is now widely recognized as a powerful tool in biocontrol (Mishra and Bohra*,* 2005).

As a soil-dwelling rhizobacterium, naturally present in the immediate vicinity of plant roots, *B. subtilis* is able to maintain stable contact with higher plants and promote their growth. In addition, due to its broad host range, its ability to form endospores and produce different antibiotics with a broad spectrum activity, *B. subtilis* as well as other members of the *Bacillus* genus can serve as potentially useful biocontrol agents (Li *et al.,* 2012).

The potential of *Bacillus subtilis* to produce antibiotics has been recognized over 50 years and were used as fungicide in agriculture and horticulture. Several *B. subtilis* strains have the potential to produce more than two dozen antifungal substances with an amazing variety of structures in which peptide antibiotics represent the predominant class. It has a potential use as an environmentally safe agent for control of crop disease (Stein, 2005). Considering the multiple applications of *Bacillus* strains, it is essential to develop efficient strains in field conditions. The research on the concept of *Bacillus* diversity studies from rhizospheric regions of different crop systems of India has been reported recently (Kumar *et al*., 2011, Gopalakrishnan *et al*., 2011).

With this background information, the present investigation on soil borne pathogens of Stevia was undertaken with the following objectives to evolve a PGPR bioformulation against fungal diseases under greenhouse conditions. The specific objectives of the present study are

- Isolation and characterization of antagonistic bacteria from rhizosphere through morphological, biochemical and molecular tests.
- *In vitro* screening of *P. fluorescens* and *B. subtilis* isolates against S. rolfsii
- *In vitro* screening of *P. fluorescens* and *B. subtilis* isolates against *A. alternata*
- Analysis of secondary metabolites such as Hydrogen cyanide (HCN), Siderophores, Salicylic acid, Indole acetic acid (IAA) and chitinase in PGPR
- Development of talc-based bioformulations of PGPR

- Evaluation of the efficacy of PGPR bioformulations against *Sclerotium rolfsii* root rot and Alternaria leaf spot of *S. rebaudiana* under greenhouse condition

- Effect of rhizobacteria on the induction of defense related compounds in *S. rebaudiana* plants

An assemblage of the background information available in the literature relavant to the present study is reviewed in the next chapter