Abstract

Plants are associated with various micro-organisms, understanding the role of the plant associated microbes and their interaction with the host has been an important topic of research for scientists. Endophytes are microorganisms that spend at least parts of their life cycle inside plants without exhibiting any pathogenic consequences to the host. It is found out that several endophytes can exhibit antagonistic activity against plant pathogens and possibly plant uses these endophytes as a weapon to fight against pathogens and protect the host from different biotic and abiotic stress. It is hypothesized that plants might carry some of these endophytes from generation to generation in order to fight against some pathogens. Among others, bacterial endophytes are one of the most studied endophytic communities that have been found to be of great importance. Endophytes facilitate the acquisition of essential nutrients such as nitrogen, phosphorus, iron etc., and also modulate level of different hormones including auxins, cytokinin and gibberellins in a host plant. The biomolecular and physiological ploy, adopted by the endophytes to establish an association with the host plant; discerning of the endophyte as non-pathogenic by the host; the crucial factors of endophytic-localization and maintenance of microbial load in the plants' internal tissues; interaction of bacterial endophytic species with other microbial communities inside the host; precise communication of microbial load and the host's developmental stage are important area of research in plant-health research which yet has been properly understood. Further, the proposition that various plants carry endophytes through seeds from generation to generation to protect themselves from pathogens is a recent hypothesis. These above intricacies have made endophytes an exciting topic of research. Among others, bacterial endophytes are one of the most studied endophytic communities that have been found to be of great importance. Bacterial endophytes are known to exhibit various activities in host plants including plant growth promotion, and seedling emergence, and also it has been reported by various researchers that bacterial endophytes provide resistance against plant pathogens and environmental stress. It has been reported that several bacterial endophytes reside in the same niche similar to plant pathogens, which might help them to be suitable bio-control agents.

Plants encounter different pathogenic microbes those causes' different diseases by various mode of infection. Some of these pathogens can enter the internal tissues of the host plant, but unlike the endophytes these grow rapidly and hijack the

plant to cause severe diseases. *Ralstonia solanacearum* is one such bacterial phytopathogen which is a soil borne bacterium that causes several bacterial wit diseases in various plants including some economically important plants like tomato, potato, brinjal, chilli, etc. Albeit, different methods have been applied to control the disease caused by the pathogen, none of them are found to be adequate yet. *R. solanacearum* F1C1 strain was originally isolated from a wilted chili plants in Tezpur, Assam, India. Previously, different methods were developed to study the pathogenicity of F1C1 strain in tomato and brinjal seedlings. The strain has been found to cause disease in seedling stages of tomato and brinjal in gnotobiotic condition. The seedling model has been recently studied by different research groups for plant-microbe interaction and bio-control efficacy of microbes against plant pathogen like *R. solanacearum*. Based on the summary, this study has been carried out to investigate the role of bacterial endophytes present in the tomato seedlings grown in controlled environment against the pathogenic F1C1 strain, and to study the efficacy of the isolates in controlling the disease caused by the pathogen.

This Ph.D. thesis has been structured in five chapters.

Chapter 1 describes a concise background of the study and research questions addressed. It also includes recent advancements corresponding to the research questions addressed.

Chapter 2 explains the role of bacteria isolated from healthy tomato seedlings germinated under controlled condition in inhibition of a bacterial phytopathogen *R. solanacearum* F1C1, isolated from wilted chilli plant. A total twenty one bacteria were isolated and were studied against F1C1 strain for their antagonistic activity. Five of the isolates were found to be exhibiting F1C1 antagonistic activity. All the twenty-one bacterial isolates were characterized for twitching motility, swimming motility and molecular characterization was done by 16S rDNA sequencing. All the five antagonistic isolates were studied for bio-control efficacy in tomato seedlings by mixing them in increasing volume with F1C1 individually and mixing the all isolates as consortia and inoculating them in the seedlings by root dip and leaf clip inoculation. All the isolates were found to have impact in reducing the disease caused by F1C1. One strain N4T belonging to *Pseudomonas putida* was studied for bio-control potential against F1C1 infection in tomato seedlings by pre-inoculating through root dip method. It was

observed when N4T was pre-inoculated; the disease caused by F1C1 was reduced. Apart from N4T pre-inoculation water was also found to play a role in reducing the disease.

In chapter 3, *P. putida* N4T strain was studied for its colonization in tomato seedlings, brinjal seedlings and grown-up tomato plants. The N4T strain was found to be able to colonize inside tomato seedlings and grown-up tomato plant also was able to colonize in brinjal seedlings. Thus, N4T was confirmed to be an endophyte. Then, it was found out that N4T was effective in reducing the disease caused by F1C1 in grown up tomato plants upon inoculating by soil drench method. Also, N4T was able to reduce the disease caused by F1C1 in brinjal seedlings upon mix leaf clip inoculation. from F1C1. Furthermore, the role of a gene *gacA* of *P. putida* N4T in the inhibition of F1C1 and C10 was studied. An insertion mutation in *gacA* homolog of N4T was created by calcium chloride ($Cacl_2$) treatment followed by heat shock method. The ability of *gacA* mutant in inhibition of F1C1 and C10 was compared by agar well diffusion assay. It was observed that the mutant strain was deficient in inhibiting C10 but it was proficient in inhibiting the pathogen F1C1. This indicates that the gene *gacA* gene has a role in inhibition of C10 but not in inhibition of F1C1.

In chapter 4, the genome of *P. putida* N4T was sequenced and analyzed using different computational tool. At first, the genome sequence was annotated and the genome was submitted in NCBI. An *in sillico* study was performed to find out different secondary metabolite gene clusters present in the genome. Then using other tool different genes was predicted for their plausible role in plant growth promoting traits. And further different genes for plant colonization and virulence were predicted using *in sillico* tool. The *P. putida* N4T genome was compared with five other *Pseudomonas* genomes in a comparative phylogenomics study.

Chapter 5 highlights the summary and future aspects of the work based on the findings of the study.