EFFICACY OF BACTERIAL HORMONE IN PLANT GROWTH PROMOTION

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IN THE NAME OF ALLAH, THE MOST MERCIFUL,
THE BENEFICIENT

DEDICATED

TO

MY PARENTS

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LIST OF ABBREVIATIONS

PGPR	Plant growth promoting rhizobacteria
PGPB	Plant growth promoting bacteria
rDNA	Ribosomal DNA
IPyA	Indole pyruvic acid pathway
LC-MS	Liquid Chromatography-Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
Col-0	Columbia-0
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
NAA	Naphthalene acetic acid
2, 4-D	2,4-Dichlorophenoxyacetic acid
MS	Murashige and Skoog
$HgCl_2$	Mercuric Chloride

SUMMARY

Plant growth promoting rhizobacteria are the plant associated bacteria that exert beneficial impact on plant growth. Bacterial auxin production is considered as an important factor responsible for phytostimulation by PGPR. The present work is concerned with the isolation of auxin producing bacterial strains from the indigenous environment and the evaluation of their auxin producing potential to affect plant growth with special emphasis on plant root system. Thirty bacteria strains were isolated from the rhizosphere, rhizoplane and histoplane of different plants. These isolates were purified and on the basis of their auxin production potential, thirteen strains were selected for further study [illegible] five already isolated auxin producing bacterial strains. Most of the strains were gram-negative rods with an optimum temperature of 37°C. The phylogenetic affiliations of the isolates were determined through 16S rDNA sequencing. Most of the bacterial strains were found to belong to the genus *Enterobacter* (A3E, A5C, A7B, A9G, AM10, A11E, A12G) while five isolates AAL1, P4, S6, AB8 and AMP2 were identified as *Bacillus*. The isolates AL2, A13G, APA, AHA, AST and AHT have shown maximum homology with *Cronobacter muytjensii, Exiguobacterium* sp., *Halomonas venusta, Arthrobacter mysorens, Halomonas* sp. and *Kushneria avicenniae* respectively. Auxin production by these isolates was primarily determined through colorimetric analysis. On the basis of colorimetric analysis, *Enterobacter cloacae* A9G was found to produce maximum amount of auxin. Variation in the cell density as well as the concentration of precursor i.e., L-tryptophan was found to affect auxin production potential of the isolates. Significant positive correlation ranging from $r = 0.864$ ($p = 0.05$) to $r = 0.983$ ($p = 0.01$) was observed between the concentration of precursor and amount of auxin produced. Auxin production by the isolates was also analyzed through Liquid Chromatography- Mass Spectrometry (LC-MS). The isolates *Halomonas venusta* APA, *Enterobacter cloacae* AM10, *Enterobacter* sp. A5C, *Enterobacter cloacae* A9G and *Enterobacter* sp. A12G were found to produce high concentrations of auxin i.e., 21.4 μgml^{-1}, 18.1μgml^{-1}, 17.5μgml^{-1}, 16.7μgml^{-1}, 16.4μgml^{-1} respectively. IAA is known to affect most of the metabolic processes by affecting the root architecture of the plants. To evaluate the effect of bacterial auxin on plant root system, *Arabidopsis thaliana*, a model plant was used. Wild type Col-0 and mutant lines *axr1-3* (auxin response mutant) and *pdr9-1* (auxin transport mutant) were used in our experiments and impact

of various synthetic auxins IAA, NAA and IBA as well as bacterial auxins produced by the selected bacterial strains (*Enterobacter* sp. A5C, *Enterobacter cloacae* A9G; *Enterobacter cloacae* AM10; *Enterobacter* sp. A12G; *Halomonas venusta* APA) producing higher concentrations of auxins, on primary root growth and lateral root development was determined. Exogenous application of synthetic auxins i.e., IAA, NAA and IBA in varying concentrations upto 1000nM resulted in the inhibition of primary root growth with simultaneous increase in the initiation and emergence of lateral roots. IAA was found to be the most effective auxin inhibiting primary root growth and causing prominent increases in the initiation and emergence of lateral roots while IBA proved to be the least effective auxin. Comparison of the affects of synthetic auxin IAA, NAA and IBA on wild type Col-0 and mutant lines *axr1-3* (auxin response mutant) and *pdr9-1* (auxin transport mutant) showed the important role played by auxins in inhibiting primary root growth as no such response was observed in auxin response mutants with reduced auxin sensitivity. *Arabidopsis thaliana* wild type Col-0 and auxin mutant lines *axr1-3* and *pdr9-1* were also grown in the presence of varying concentrations of bacterial IAA (A5C, A9G, AM10, A12G, APA) and responses observed were comparable to those observed with synthetic IAA. The auxin response mutant *axr1-3* exhibited reduced sensitivity to bacterial IAA (A5C, A9G, AM10, A12G, APA). *pdr9-1* is an auxin transport mutant affecting the transport of 2,4,-D and exhibiting resistance to IBA. Inhibition of primary root growth and stimulation of lateral root initiation and emergence observed in *pdr9-1* in response to bacterial auxins (A5C, A9G, AM10, A12G, APA) was comparable to the response of *pdr9-1* observed with synthetic IAA confirming that the bacterial auxin is IAA in nature. Transgenic line DR5::GUS are the auxin reporter constructs with auxin response element fused to the reporter gene and are sensitive to auxin treatment. In DR5:: GUS, auxin signaling is generally observed in response to auxin treatment. Strong GUS activity was recorded in response to bacterial IAA comparable to the GUS signals observed with synthetic IAA. Maximum GUS expression was recorded in response to bacterial IAA produced by *Halomonas venusta* APA. Growth stimulatory impact of auxin producing isolates was observed in plant-microbe interaction experiments using *Solanum tuberosum* and *Hibiscus esculentus*. Most of the isolates caused increases in the growth parameters. It was observed that the phytostimulatory effects were not source specific. However, among the isolates, bacterial strains belonging to the genera *Enterobacter* and *Bacillus* were found to have phytostimulatory potential greater than the rest of the isolates. Bacterial IAA from two of the

isolates AM10 and A5C was also successfully utilized to induce callogenesis in *Brassica oleraceae*. Hence these auxin producing bacterial strains proved to be a cheap source of IAA and can be utilized efficiently for the plant growth promotion in a cost effective manner.

Chapter 1

INTRODUCTION

Plants coordinate and control their development by using chemical signals to regulate the growth of cells throughout the plant. These signals called phytohormones have profound effects on development at vanishingly low concentration and have the ability to alter plant growth patterns. These natural phytoregulators are synthesized by the plant itself and act in a location distant from the source of production (Avanci *et al.*, 2010). According to the classical view, there are five classes of phytohormones i.e., auxins, cytokinins, gibberellins, ethylene and abscissic acid (Savaldi-Goldstein *et al.*, 2008) but there is increasing evidence of other compounds to have shown growth regulating activities in plants. Among these are brassinosteroids, jasmonates, salicylic acid, strigolactones, etc. (Khripach *et al.*, 2000; Tanimoto, 2005; Ruzicka *et al.*, 2007; Michael *et al.*, 2008; Avanci *et al.*, 2010; Rameau, 2010; Taiz and Zeiger, 2010). Auxins, cytokinins, gibberellins and brassinosteroids promote shoot growth while others including ethylene, abscissic acid and jasmonates control growth activities by regulating growth inhibitory processes in plants such as dormancy, abscission, senescence etc.

Auxin is the most extensively studied phytohormone that seems to function as a master control system, regulating many aspects of the overall biology of the plants by controlling diverse plant processes (Tanimoto, 2005; Smith, 2008; Donner *et al.*, 2010). A continuous flow of auxin is produced by the growing tip of the shoot that travels down the plant stem and into the roots. This flow creates a chemical gradient inside the plant that regulates the growth of lateral shoots and roots and so determines the overall shape and structure of a plant. Auxins are most abundantly produced in meristematic areas e.g., root and shoot tips but are also present elsewhere e.g., in the stems and leaves (Zazimalova and Napier, 2003; Hopkins and Huner, 2004). Plant growth is found to be suppressed by decapitation or by the application of auxin transport inhibitor N-1-naphthylphthelamic acid (NPA) indicating the production of auxin in the growing points of plant (Wu and McSteen, 2007; Ferguson and Beveridge, 2009). Growth is found to be restored by IAA application mixed with lanolin confirming that growth is controlled by auxin mostly supplied from the apical region. Growth has a positive correlation with the level of endogenous IAA (Strachan, 2003). Transport and metabolism are the key factors