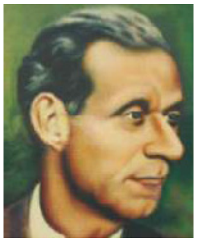

Coleman Lecture 2017

The 'Coleman Lecture' series was instituted in 2014 by the Departments of Entomology and Plant Pathology, UAS, Bangalore in honour of Dr. Leslie Coleman, who was appointed as the first State Mycologist in 1908.

Dr. Coleman pioneered research in plant protection in the then Mysore State Department of Agriculture.

The lectures are held on 16 June of every year - the birthday of Dr. L. C. Coleman.

The Fourth Coleman Lecture was held this year under the aegis of the Dean (Post-Graduate Studies), UAS, Bengaluru.

Fourth Coleman Lecture

Plant endophytic bacteria: modulators of plant immunity and reservoir of novel biofunctional compounds-

delivered by

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Plant endophytic bacteria: Modulators of plant immunity and untapped reservoir of novel biofunctional compounds

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Introduction

Plants associated niches are one of the important habitats for microorganisms that colonize epiphytic and endophytic spaces. Endophytic microbes are unique as they live intimately with plants while contributing towards plant growth, development and innate immunity. Endophytes, especially bacteria have been shown to prevent disease development through endophyte-mediated *de novo* synthesis of unexplored antimicrobial compounds and induction of systemic resistance. Plant growth promoting endophytes differ from biocontrol strains in that they do not necessarily inhibit pathogens but increase plant growth through the improved cycling of nutrients and minerals such as nitrogen, phosphate and other micronutrients. Some of the key benefits of endophytic bacteria for plants are narrated below.

Plant-Endophyte interaction

Endophytic bacteria are known to interact with different plant cells in a programmed manner. Soil released *Pseudomonas aeruginosa* BP35 tagged with reporter gene, green fluorescent protein, could be tracked in leaf and other aerial portion within two weeks of release indicating active migration within the plant system. Interestingly, the endophytic bacterium is found to colonize taxonomically distant plant species. Black pepper endophytic bacterium *Pseudomonas putida* BP25 not only colonized it host, black pepper, but also other plant species such as *Zingiber officinale* and *Arabidopsis thaliana* Col 0. *Pseudomonas aeruginosa* BP35 was found colonized tomato leaf upon artificial inoculation (Kumar et al 2013). The current understanding of plant microbe interaction suggest that the microbes are perceived by plants receptors termed as Pattern Recognition Receptor (PRR) by highly conserved microbial signatures termed as Microbe Associated Molecular Pattern (MAMP). Interaction between PRR and MAMP leads to cascades of signaling events in plant cytoplasm which culminate in several defense linked plant phenotypes that is, by and large, intangible.

Endophytic bacteria triggers plant innate immunity

Endophytic microbes do interact with plant cells in a well-coordinated manner leading to modulation of hormonal signaling as observed in *Arabidopsis thaliana* Col 0-*Pseudomonas putida* BP25. Endophytic *P. putida* BP25 triggered density dependent alterations on *A. thaliana* Col-0 growth and development. Endogenous colonization of *P. putida* BP25 was found regulated within *Arabidopsis* that caused induction and repression of 131 and 74 plant genes, respectively. Induced genes like *WRKY33, AtRLP19, ATL2, ATEXO70B2, pEARLI, RPS2, CBP60G, PLA2, CRK18, ATFBS1, DREB2A, TIR, RAP2.4,* and *MOS1* were components of defense and salicylic acid (SA) signaling. Plant growth and development associated genes were found significantly repressed. Biased activation of phytohormone signaling with their associated fitness costs on plant growth was also observed in arabidopsis. Many defense related genes such as *MYB7, MYB4, MYB49, WRR4, ATHCHIB* and *ATOSM34* were found up-regulated in *Arabidopsis* upon colonization by another endophytic *Bacillus megaterium* BP17. The data suggests that endophytic bacterial colonization triggered expression of defense genes that restricted its own population besides causing altered root phenotype which indicated enhanced plant immunity (Sheoran et al 2016).

Endophytic bacteria triggers plant growth

Another endophyte, *B. megaterium* BP17 showed excellent growth promotion in plant. Several plant genes participation in nutrient utilization was found up regulated in plants. *Bacillus megaterium* BP17 endophytically colonized plantlets of *Arabidopsis* with significant growth promotion as exemplified by increased root and shoot length. Microarray based gene expression profiling of interactome of *Arabidopsis thaliana*-*Bacillus megaterium* BP17 revealed differential expression of over 150 genes representing 80 induced and 70 repressed plant genes. Some of the up-regulated genes were i. *NIR1, AMT1-3, SULTR1-2, SHV3, MMP, RLP44, PROPEP4, AGL42, SCPL30, TIP2-3, ANAC010* and *KNAT7* participating in growth and developmental processes, transport of nutrient through transmembranes, cell organization, biogenesis and transcription; and ii. *SRO5, ANNAT7* and *DDF1* associated with tolerance to abiotic stresses like salt stress and water deprivation. The up-regulation of nutrient uptake related genes could be involved in plant growth promotion. The bacterial colonization triggered down-regulation of genes coding for transcription factors of ethylene responsive genes such as *ERF5, ERF71, ERF104, TEM1, RAV2* and *RAP2.6* and genes participating in response to salicylic acid and jasmonic acid such as *BAP1, SIB1, BT4, PLA2A* and *MKK9*. This study showed that the plant growth promotion as observed in *A. thaliana* Col 0 could be attributed to endophytic bacteria mediated up-regulation of several growth and development associated genes (Vibhuti et al 2017).

Endophytic bacteria releases antimicrobial compounds

Antimicrobial compounds especially antibiotics are long known to be produced by microorganisms. Biochemical pathways for synthesis of antimicrobial compounds have been deciphered (Song et al 2014; Song et al 2015). Bacterial species isolated from black pepper endosphere belong to *Pseudomonas aeruginosa, Pseudomonas putida, Bacillus megaterium,* and *Curtobacterium luteum* displayed good antagonism against range of plant pathogens including nematodes (Aravind et al 2009; Aravind et al 2010; Aravind et al 2012). Studies on mechanisms of antagonism revealed that *P. putida* BP25 and *B. megaterium* BP17 released antimicrobial volatiles belong to pyrazines and sulfur containing compounds like dimethyl tri sulphide (Sheoran et al 2015; Vibhuti et al 2016). Dynamic head space GC/MS analysis of airborne volatiles indicated the presence of aromatic compounds such as 1-Undecene; Disulfide dimethyl; Pyrazine, methyl- Pyrazine, 2,5-dimethyl-; Isoamyl alcohol; Pyrazine, methyl-; and Dimethyl trisulfide in *P. putida* BP25. Volatiles of *B. megaterium* BP17 consisted of Pyrazine, 2-ethyl-3-methyl-; Pyrazine, 2, 5-dimethyl-; Pyrazine, ethyl-; and Pyrazine, methyl-. These bacterial origin molecules showed excellent inhibition of pathogens belong to diverse taxonomic groups (Sheoran et al 2015; Vibhuti et al 2016).

Not all endophytic bacteria are safe for agricultural use

While several endophytic bacteria showed promise for commercialization as bio-inoculant, there are few that displayed undesirable bacterial traits failing in biosafety trials in animal testing experiments. One of our excellent antagonistic endophytic bacteria, *P. aeruginosa* BP35 isolated from aerial shoots of black pepper grown in the rain forest of the Western Ghats in Kerala, India contributed significant protection to black pepper against infections by *Phytophthora capsici* and *Radopholus similis*. For registration and implementation in disease management programs, several genetic and phenotypic traits of *P. aeruginosa* BP35 were investigated including its endophytic behavior, biocontrol activity, phylogeny and toxicity to mammals. The results showed that *P. aeruginosa* BP35 efficiently colonized black pepper shoots and displayed a typical spatiotemporal pattern in its endophytic movement with concomitant suppression of *Phytophthora* rot. Confocal laser scanning microscopy revealed relatively high populations of *P. aeruginosa* BP35::*gfp2* inside tomato plantlet tissues, supporting its endophytic behavior in other plant species. Polyphasic approaches to genotype *P. aeruginosa* BP35, including BOX-PCR, *rec*N sequence analysis, Multilocus Sequence Typing (MLST) and Comparative Genome Hybridization (CGH) analysis, revealed its uniqueness among the global collection of *P. aeruginosa* strains isolated from other environmental and clinical habitats (Kumar et al 2013). However, like other *P. aeruginosa* strains, *P. aeruginosa* BP35 exhibited resistance to multiple antibiotics, grew at 25-41°C and produced rhamnolipids, HCN and phenazines. *Pseudomonas aeruginosa* BP35 displayed strong cytotoxicity on mammalian A549 cells indicating its proficiency in type II secretion effectors. Coupled with pathogenicity in a murine airway infection model, it is concluded that this plant endophytic strain is as virulent as clinical *P. aeruginosa* type strains (Kumar et al 2013)

Future of endophytic bacterium

Bacterial endophytes are reported to protect the plants from pathogens by production of antibiosis, out competition of pathogens and induced systemic resistance. Endophytic bacteria assisted crop protection and the eventual enhanced crop production requires careful selection of candidate microorganism with antagonistic potential against broad range of plant pathogens including nematodes. Other additional benefits such as nutrient solubilization and plant growth promotion, if available with the microbes, can also be exploited. Due emphasis must be given to biosafety as several plant associated bacteria are potential & opportunistic human pathogen. An ideal endophyte touted as bioinoculant is the one that possesses broad spectrum activity against range of biotic stress factors without any genetic link with clinical origin microbes. Plant endophytic bacteria can also be used as biological resource for isolation and utilization of bacterial metabolites, MAMPs and other defense elicitors for imparting general immunity against broad range of pathogens.

Acknowledgements

Speaker is grateful to Department of Biotechnology, Ministry of Science and Technology, New Delhi for funding projects on endophytic bacteria. Speaker is also thankful to Director, IARI, New Delhi for providing all infrastructure facilities.

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