6 SUMMARY

1. Ten representative locations were selected in two taluks, viz., Chidambaram and Kurinjipadi at Cuddalore district, Tamil Nadu state, India to survey the occurrence of *Azospirillum*, *Pseudomonas* and *Methylobacterium* from the rhizosphere and phyllosphere, respectively, of lowland rice.

   A range of 0.85 to 1.23 per cent of *Azospirillum* population, and a range of 1.14 to 1.62 per cent of *Pseudomonas* population to the total bacterial population was observed in the survey whereas it was 0.66 to 1.02 per cent for *Methylobacterium* in the phyllosphere of lowland rice. The location, Sathamangalam of Chidambaram taluk recorded the highest population for *Azospirillum*, *Pseudomonas* and *Methylobacterium* (1.23, 1.62 and 1.02 per cent, respectively, to the total bacterial population) whereas the location, Killai of Chidambaram taluk recorded the lowest incidence of *Azospirillum*, *Pseudomonas* and *Methylobacterium* (0.85, 1.14 and 0.66 per cent, respectively, to the total bacterial population). The community population of *Azospirillum* observed in ten locations ranged at $10^4$ to $10^6$ CFU g$^{-1}$ of rice rhizosphere soil whereas it was $10^4$ to $10^6$ CFU g$^{-1}$ of rhizosphere for *Pseudomonas*. Interestingly, the community phyllosphere population of *Methylobacterium*, observed in the ten locations ranged from $10^4$ to $10^5$ CFU g$^{-1}$ of leaf sample.

2. Each ten strains of *Azospirillum*, *Pseudomonas* and *Methylobacterium* were isolated from the rhizosphere and phyllosphere samples of rice, collected from the ten locations of Cuddalore district, and designated as AZ-1 to AZ-10, PF-1 to PF-10 and MB-1 to MB-10, respectively.
Occurrence of *Azospirillum*, *Pseudomonas* and *Methylobacterium* bacterial genera in the rice rhizosphere or phyllosphere grown at Cuddalore district, Tamil Nadu state, India was confirmed.

3. Two *Azospirillum* isolates (20 per cent of the total isolates) recorded the 'N' fixation above 15 mg 'N' fixed g\(^{-1}\) of malate, five isolates (50 per cent of the total isolates) recorded the N-fixation in a range of 14.00 to 14.99 mg ‘N’ fixed g\(^{-1}\) of malate while the remaining isolates (30 per cent of the total isolates) recorded the N-fixation below 14 mg ‘N’ fixed g\(^{-1}\) of malate. The isolate, AZ-3, an isolate from Sathamangalam of Chidambaram taluk, recorded a maximum of 15.75 mg of ‘N’ fixed g\(^{-1}\) of malate and all other isolates showed the N-fixation in the range of 12.84 to 15.58 mg ‘N’ fixed g\(^{-1}\) of malate.

4. Two *Pseudomonas* isolates (20 per cent of the total isolates) recorded the IAA production and zone of inhibition as >4.80µg mL\(^{-1}\) and 10.00 mm dia. respectively, five *Pseudomonas* isolates (50 per cent of the total isolates) recorded the IAA production and zone of inhibition in a range of 4.00 to 4.79 µg mL\(^{-1}\) and 9.00 to 9.99 mm dia. respectively while the remaining *Pseudomonas* isolates (30 per cent of the total isolates) recorded IAA production and zone of inhibition as <4.00 µg mL\(^{-1}\) and <9.00 mm dia. The isolate, PF-3, an isolate from Sathamangalam of Chidambaram taluk, recorded a maximum of 5.02 µg mL\(^{-1}\) IAA production and 11.02 mm dia. of zone of inhibition against *Xanthomonas oryzae pv oryzae* and all other isolates showed IAA production and zone of inhibition in a range 3.23 to 4.82 µg mL\(^{-1}\) and 8.13 to 10.91 mm dia. zone of inhibition, respectively.
5. Two *Methylobacterium* isolates (20 per cent of the total isolates) recorded the IAA production and zone of inhibition as >2.75µg mL\(^{-1}\) and 8.70 mm dia. respectively, five *Methylobacterium* isolates (50 per cent of the total isolates) recorded the IAA production and zone of inhibition in a range of 1.50 to 2.74 µg mL\(^{-1}\) and 8.00 to 8.69 mm dia. respectively while the remaining *Methylobacterium* isolates (30 per cent of the total isolates) recorded IAA production and zone of inhibition as <1.50 µg mL\(^{-1}\) and <8.00 mm dia. The isolate, MB-3, an isolate from Sathamangalam of Chidambaram taluk, recorded a maximum of 2.96 µg mL\(^{-1}\) IAA production and 8.83 mm dia. of zone of inhibition against *Xanthomonas oryzae pv oryzae* and all other isolates showed IAA production and zone of inhibition in a range 1.32 to 2.82 µg mL\(^{-1}\) and 7.51 to 8.72 mm dia. zone of inhibition, respectively.

6. The two selected efficient *Azospirillum* isolates which recorded ‘N’ fixation above 15 mg ‘N’ fixed g\(^{-1}\) of malate viz., AZ-3 and AZ-6, two selected efficient *Pseudomonas* isolates which recorded IAA production >4.80 µg mL\(^{-1}\) and zone of inhibition >10 mm dia. viz., PF-3 and PF-6 and the two efficient *Methylobacterium* isolates which recorded IAA production above 2.75 µg mL\(^{-1}\) and biocontrol ability against *Xanthomonas oryzae pv oryzae* (>8.70 mm dia. inhibition zone) viz., MB-3 and MB-6 were selected and examined for interstrain differences, such, as growth characteristics under N-free and N-supplementation conditions, acetylene reduction activity (ARA), IAA, EPS and siderophore production, adhesion to rice roots and desiccation and thermal tolerance. Interstrain differences were found to be existed among the tested isolates for the above mentioned characters. The
Azospirillum, Pseudomonas and Methylobacterium isolates, viz., AZ-3, PF-3 and MB-3, respectively, exhibited higher performance for all these characters whereas the isolates, AZ-6, PF-6 and MB-6 exhibited the poor performance.

7. The speciation of efficient Azospirillum (AZ-3), Pseudomonas (PF-3) and Methylobacterium (MB-3) isolates, on the basis of the phenotypic characters, revealed that the efficient Azospirillum isolate, viz., AZ-3, belonged to the species brasilense, efficient Pseudomonas isolate viz., PF-3, belonged to the species fluorescence whereas the efficient Methylobacterium isolate viz., MB-3, belonged to the species phyllosphaerae.

8. The factors affecting the multigenic co-aggregation of efficient Azospirillum (AZ-3), Pseudomonas (PF-3) and Methylobacterium (MB-3) cells, revealed that $10^7:10^7:10^7$ inoculum level of PGPB partners was found to be optimum for the maximization of multigenic co-aggregation while any increase or decrease to this inoculum level could reduce the co-aggregation percentage of the same.

9. The use of Azospirillum, Pseudomonas and Methylobacterium cells at stationary growth phase augmented the multigenic co-aggregation to a higher level when compared to the lag and log phase of the PGPB cells and suggested that the cell surface modification of the PGPB cells during stationary growth phase might be the reason.

10. The Azospirillum, Pseudomonas and Methylobacterium cells harvested from N-deficient medium yielded more multigenic co-aggregation percentage when compared to the cells harvested from N-supplemented
medium. Higher EPS production by the PGPB cells in N-deficient medium might be the reason for higher multigeneric co-aggregation.

11. The *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells, grown at 35°C temperature level, augmented more multigeneric co-aggregation among PGPB cells and any increase or decrease to this temperature level could reduce the co-aggregation processes. The alteration in the composition of outer membrane protein (OMP) of PGPB cells due to changing level of temperature might play a determining role in the co-aggregation.

12. The use of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells recorded a low level of co-aggregation at pH 7.0 and any increase or decrease to this pH level caused more multigeneric co-aggregation of PGPB cells. The multigeneric co-aggregation of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells was stable at >7.5 and <6 pH levels with dispersion at pH 7.0.

13. Addition of Ca$^{2+}$, as divalent cation, to the co-aggregation buffer could augment the multigeneric co-aggregation of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells to a higher level when compared to the addition of other divalent cations, viz., Mg$^{2+}$ and Ba$^{2+}$.

14. Addition of EDTA, as chelating agent, to the co-aggregation buffer could reduce the multigeneric co-aggregation of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells to a significant level when compared to EGTA. The observation clearly envisaged the role of protein in cell-to-cell interaction.

15. Addition of *Moringa oleifera* seed materials, as plant seed flocculant, inducted a higher level of artificial multigeneric co-aggregation among
Azospirillum, Pseudomonas and Methylobacterium cells at log phase of growth followed by Strychnos potatorum, Allium cepa, Sappindus emarginatus and Asteracantha longifolia.

16. The thermal and desiccation tolerance of multigeneric PGPB co-aggregates (natural) were found to be more when compared to PGPB multigeneric coaggregates (artificial) inducted by Moringa seed materials. Accumulation of higher level of cellular reserve materials of multigeneric PGPB co-aggregates (natural) might be the reason for the same.

17. The application of Azospirillum, Pseudomonas and Methylobacterium cells, as natural multigeneric co-aggregates, could augment the seed vigour index, adhesion to rice root and reduction in Xanthomonas oryzae pv oryzae incidence in rice when compared to the application of multigeneric PGPB co-aggregates (artificial), co-inoculation and single strain inoculation of Azospirillum, Pseudomonas and Methylobacterium.

18. Exogenous application of salicylic acid (SA) was found to control the bacterial leaf blight (BLB) disease of rice crop efficiently and provide indirect evidence for the induction of systemic resistance in rice against bacterial leaf blight pathogen (Xanthomonas oryzae pv oryzae).

19. The application of EPS rich, natural multigeneric PGPB co-aggregates application, consisting of Azospirillum, Pseudomonas and Methylobacterium cells together with salicylic acid application and challenge inoculation of Xanthomonas oryzae pv oryzae exerted both phytostimulatory and biocontrol effect against Xanthomonas oryzae pv oryzae in rice to a higher level when compared to other PGPB bioformulations viz., natural co-aggregates alone, artificial co-aggregates
+ Salicylic acid, artificial co-aggregates alone, co-inoculation + Salicylic acid, co-inoculation alone, single strain + Salicylic acid and single strain alone treatments.

20. The application of EPS rich, multigeneric natural PGPB co-aggregates application, consisting of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells, together with supplementation of salicylic acid and challenge inoculation of *Xanthomonas oryzae pv oryzae* augmented the ISR of rice cv. BPT-5804 plant by increasing the total phenolic content, reduction in reducing and non reducing sugar level and increased the starch content of the host plant. Moreover, the application of the same bioformulation augmented the host defense enzymatic activities viz., peroxidase (PO) and polyphenol oxidase (PPO), to a maximum level when compared to other bioformulations supplemented with or without salicylic acid viz., natural co-aggregates alone, co-inoculation with salicylic acid, co-inoculation alone, vegetative cells with salicylic acid and vegetative cells alone.

21. The application of EPS rich, natural multigeneric PGPB co-aggregates application, consisting of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells, together with supplementation of salicylic acid and challenge inoculation of *Xanthomonas oryzae pv oryzae* at 75 per cent recommended dose of ‘N’ nutrition augmented the growth parameters of rice cv.BPT-5804 viz., height, dry matter production, organic carbon content, nitrogen content, IAA production, chlorophyll content and bacterial leaf blight disease incidence and yield parameters viz., grain and straw yield to a higher level followed by natural multigeneric PGPB co-aggregates alone, co-inoculation with salicylic
acid, co-inoculation alone, single strain with salicylic acid and single strain inoculation of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells and control at 100 per cent recommended ‘N’ application without any bioinoculation.

The application of “Multigeneric PGPB co-aggregates, consisting of *Azospirillum*, *Pseudomonas* and *Methylobacterium*” cells under natural condition together with supplementation of salicylic acid (0.1 per cent) and challenge inoculation of *Xanthomonas oryzae pv oryzae* at 75 per cent recommended dose of ‘N’ levels in rice cv.BPT-5804 recorded the growth and yield parameters on par with 100 per cent recommended ‘N’ nutrition level application without any bioinoculation treatment and thus a saving of 25 per cent recommended ‘N’ nutrition level could be achieved through the application of “Multigeneric PGPB co-aggregates (natural)” in lowland rice crop. Moreover, the natural multigeneric co-aggregates application augmented the survival of PGPB cells in rice rhizosphere and phyllosphere and afforded an ISR-mediated biocontrol against the bacterial leaf blight disease phytopathogen (*Xanthomonas oryzae pv oryzae*) of lowland rice and thus preventing the biological and environmental hazards posed by the persistent use of synthetic chemical fertilizers and pesticides and improve the productivity of rice crop under lowland condition.

**FUTURE PERSPECTIVES OF THE STUDY**

The results of the present study clearly confirmed the positive role of EPS rich, natural multigeneric PGPB co-aggregates, consisting of *Azospirillum*, *Pseudomonas* and *Methylobacterium* cells along with salicylic acid application on plant growth stimulation and ISR mediated biocontrol against *Xanthomonas*
oryzae pv oryzae in lowland rice crop. This is a preliminary research and it needs further exploitation on the components and composition of EPS obtained from the natural multigeneric PGPB co-aggregates, which is highly essential and responsible for the induction of systemic resistance in rice plant. Moreover, the biochemical, physiological and molecular aspects of “EPS mediated ISR in rice plant” against bacterial leaf blight pathogen *viz.*, *Xanthomonas oryzae pv oryzae* need to be investigated.

Further, more cytological and cytochemical studies are needed to exploit the relationship between microbial co-aggregates and their EPS which helps in the commercial exploitation of “Multigeneric PGPB co-aggregates”, as a novel agricultural bioinoculant, for the maximization of rice productivity under lowland condition.