5. DISCUSSION

Rice (*Oryza sativa* L.) is the staple food of more than 60 per cent of the world’s population especially for the most of the people in South East Asia and India. Among the rice growing countries in the world, India has the largest area under rice crop and ranged second in the production, next to China. In India, rice is grown under both upland and lowland conditions and out of 44 million hectare of rice cultivated area, 12 per cent of the same grown under lowland condition. However, the production of lowland rice has been volatile and the yield of the same is affected by a lot of biotic and abiotic factors, including, ‘P’ nutrition, temperature and water stress and disease incidence. Phosphorus is one of essential nutrient required by both plants and microorganisms. Phosphorus is generally deficient in lowland rice soils and fixed as water insoluble calcium phosphate and eventually lead to the reduction in biological nitrogen fixation. Generally, large quantities of synthetic chemical fertilizers and pesticides are used to replenish the same, resulting in high costs and severe environmental and biological hazards. Recently, a biological approach of using plant growth promoting rhizobacteria (PGPR) was attempted to reduce the drastic effects caused by persistent use of synthetic chemical fertilizers and pesticides and to improve the productivity of lowland rice crop. Moreover, PGPR mediated Induced systemic resistance (ISR) against phytopathogen seems to be a promising approach in the reduction of biological and environmental hazards posed by the application of synthetic chemical pesticides. In the last few years, several new formulations of agricultural bioinocula have been tried with an aim to improve the shelf life of the same during stress conditions including moisture and temperature.

The positive role of fluorescent pseudomonads, as PGPR and biocontrol agent in rice ecosystem has been reported by many workers (Mew and Rosales,
1986; Ramezonpour et al., 2010; Ramezonpour, 2011 Sakthivel and Gnanamickam, 1987; Saveetha et al., 2009; Vidyasakeran and Muthamilan, 1999; Vidyasakeran, 1996). *Pseudomonas fluorescens*, as a member fluorescent pseudomonads, play a significant role in enhancing the plant growth promotion and biocontrol of many phytopathogens in rice crop. *Paenibacillus polymyxa* inhabits different niches, such as, soils, roots and rhizosphere of rice (Bacon et al., 2001; Mc Spadden Gardener, 2004; Mehta et al., 2010; Palumbo et al., 2007; Sadhana Lal and Tabacchioni, 2009; Tilak and Srinivasa Reddy, 2006). Both these organisms exert PGPR characteristics in the rhizosphere and promote the growth of rice plant. Hence, the development and deployment of these organisms, as an agricultural bioinoculant, will be the suitable biological approach for the maximization of growth and yield in rice plant (Ashraf et al., 2004; Gholami et al., 2009; Nadeem et al., 2006).

Eventhough, very few reports suggested the positive role of *Pseudomonas* and *Paenibacillus* co-inoculation in rice crop, the role of EPS-rich *Pseudomonas* and *Paenibacillus* coflocs application on the maximization of growth and yield in rice crop has not been studied, so far. Moreover, the effect of EPS rich coflocs application of *Pseudomonas* and *Paenibacillus*, as an elicitor of ISR, against phytopathogens of rice will also be elucidated. In the present study, certain aspects of “Intergeneric coflocs of *Pseudomonas – Paenibacillus* on the maximization of growth and yield of rice crop grown under lowland condition” was studied with special emphasis to 1) Improvement of the shelf life of bioinoculant during stresses prevailing under lowland condition and 2) EPS mediated elicitation of ISR against bacterial leaf blight pathogen (*Xanthomonas oryzae*) of rice.
5.1. Occurrence of *Pseudomonas* and *Paenibacillus* in rice rhizosphere

In the present study the occurrence of *Pseudomonas* and *Paenibacillus* in lowland rice crop grown at Ulundurpet and Thirukkovilur taluks of Villupuram district, Tamilnadu state, India was surveyed in 20 selected locations. The *Pseudomonas* and *Paenibacillus* cultures were isolated from all the selected locations, and the ACC-deaminase production potential of the same was studied and all the isolates were characterized. With selected *Pseudomonas* and *Paenibacillus* isolates, the PGPR characteristics *viz*., IAA and siderophore production, phosphate solubilization and Exopolypolsaccharide (EPS) production potential were assayed. After determining the efficiency for the above mentioned characters, one of the most efficient isolate from each genera, namely, *Pseudomonas* and *Paenibacillus* has been selected and used for further studies.

With the most efficient isolates of *Pseudomonas* and *Paenibacillus*, the optimization of different factors that influencing the “Intergeneric co-flocculation” of *Pseudomonas* and *Paenibacillus* was assayed. Moreover, the application effect of EPS rich, natural (N) and artificial (A) coflocs of *Pseudomonas* and *Paenibacillus* on the enhancement of seed vigour index, rice root adhesion and ISR mediated biocontrol against bacterial leaf blight disease pathogen, (*Xanthomonas oryzae*), of rice has also been studied. A pot culture study was also included to evaluate the application effect of different formulations *viz*., single strain inoculation, co-inoculation and coflocs application of PGPR cells *viz*., *Pseudomonas* and *Paenibacillus*, on the enhancement of growth and yield parameters of lowland rice at 75 per cent recommended level of ‘P’ application.
The occurrence of *Pseudomonas* and *Paenibacillus* in different niches, such as, soils, roots, and rhizosphere of various crop plants, including rice has been reported by Andrew and Harris (2000), Beattie and Lindow (1995), Gholami et al. (2009), Lindow and Brandl (2003), Mehta et al. (2010), Ramezanpour et al. (2011), Sadhana Lal and Tabacchioni (2009), and Saveetha et al. (2009). In the present study, the community population of *Pseudomonas* and *Paenibacillus* in twenty selected locations of Villupuram district, Tamil Nadu state, India was studied where rice is grown under lowland condition. The results of the present study clearly revealed the ubiquitous occurrence of *Pseudomonas* and *Paenibacillus* in lowland rice soils of Villupuram district, Tamil Nadu state, but with variation in the community population level. The community population of *Pseudomonas* was found to be more in rice rhizosphere than *Paenibacillus* population. Mew and Rosales (1986) and Ramezanpour (2011), reported the ubiquitous occurrence of *Pseudomonas* in rice rhizosphere. Guemouri–Athmani et al. (2000) and Von der Weid et al. (2000) reported the occurrence of *P. polymyxa* in rice rhizosphere. The results of the present study are in conformity with the earlier findings of Guemouri–Athmani et al. (2000), Mew and Rosales (1986), Ramezanpour (2011), and Von der Weid et al. (2000).

The results of the present study also revealed a marked variation in the community population of *Pseudomonas* and *Paenibacillus* in twenty selected locations. A range of 1.15 to 1.88 per cent of *Pseudomonas* to the total bacterial population was observed in the survey whereas *Paenibacillus* recorded a range of 0.55 to 0.90 per cent to the total bacterial population. The size of the *Pseudomonas* population, expressed as per cent of total bacterial population of soil, has been reported as 1–10% by Usha Rani (2005). However, there were no
earlier reports regarding the community population of *Paenibacillus* in lowland rice rhizosphere, available.

The high incidence of *Pseudomonas* in tropical soils may be attributed to the low level of available nitrogen or to higher temperature requirements of these organisms (Ripp *et al.*, 2000; Srivastava *et al.*, 1999 Turnbull *et al.*, 2001). In the present study, one location, namely, Thakadi was found to have the highest *Pseudomonas* population (1.88 per cent) to the total bacterial population. In the remaining 19 locations, the community population was found to range from 1.0 to 1.72 per cent to the total bacterial population. Also, the same location recorded a high incidence of *Paenibacillus* viz., 0.90 per cent to total bacterial population. In the remaining location, the *Paenibacillus* population was ranged from 0.55 to 0.85 per cent to total bacterial population. Bais *et al.* (2004) and Whipps (2001) reported the low *Paenibacillus* population in lowland rice soil than *Pseudomonas* species. Weller (1988) suggested the occurrence of *Paenibacillus* population within the range of 0.001–1.0 per cent to the total bacterial population. The results of the present study clearly revealed the predominance of *Pseudomonas* population over *Paenibacillus* population in lowland rice soils of Villuppuram district, Tamilnadu, India and in conformity with the earlier findings of (Bais *et al.*, 2004; Whipps, 2001 and Weller, 1988).

The *Pseudomonas* population (CFU/g) was recorded in summer cereals of Karnataka, India upto $10^6$–$10^8$ (Rangeshwaran and Prasad, 2000) and in wheat soils upto $10^3$–$10^6$ (Hoflich, 1992; Tzang *et al.*, 1994). In the present study, the population of *Pseudomonas* observed in twenty selected locations ranged from $10^5$ to $10^6$ CFU/g of lowland rice soil. The *Paenibacillus* population (CFU/g) in summer cereals were found in a range of $10^3$ to $10^6$
(Halverson et al., 1993; Mahaffee and Kloeper, 1997; Seldin et al., 1998; Vargas–Ayala et al. 2000). In the present study, the Paenibacillus population observed in twenty selected locations ranged from $10^5$–$10^6$ (CFU/g) in lowland rice rhizosphere soil.

5.2. Isolation and characterization of Pseudomonas and Paenibacillus from rice rhizosphere

In the present study, twenty cultures of Pseudomonas (PF-1 to PF-20) and twenty cultures of Paenibacillus (PB-1 to PB-20) were isolated from the twenty locations situated in Villuppuram district, Tamilnadu state, India. The isolates were identified based upon colony characters and examination of individual cells under phase contrast microscope. All the twenty isolates (PF-1 to PF-20) exhibited the characters of Pseudomonas viz., flat, entire colonies, 0.5 cm in diameter, and diffusible fluorescent pigment after 3 to 4 days on nutrient agar plates and gram negative, motile, non-spore former (Palleroni, 1984) whereas the other twenty isolates viz., (PB-1 to PB-20) exhibited the characters of Paenibacillus viz., pale colonies on tryptic soy agar yeast extract (TSAYE) medium, rod shaped cells with 0.5–0.7 µm width and 2–5 µm length, gram positive, motile, peritrichous flagella upon prolonged incubation on agar medium, cells produced central ellipsoidal spores with swollen sporangium (He et al., 2007). Occurrence of Pseudomonas in rice soils has been reported by many workers (Ramezanpour et al., 2011; Saveetha et al., 2009) whereas the occurrence of Paenibacillus in rice soils has been reported by Ding et al. (2005), Mc Spadden Gardner (2004) and Selim et al. (2005). The results of the present study also clearly revealed the ubiquitous occurrence of both Pseudomonas and Paenibacillus in the rhizosphere soils of lowland rice grown at Tamilnadu state, India.
5.3. Screening the *Pseudomonas* and *Paenibacillus* isolates for ACC-deaminase (*acd*) activity

All the forty *Pseudomonas* and *Paenibacillus* isolates were evaluated for their ACC-deaminase (*acd*) production potential according to the method of Honma and Shimomura (1978) under *in vitro* condition. Based on the amount of ACC degradation and α-ketobutyrate production by the *Pseudomonas* and *Paenibacillus*, all the 40 isolates were made into 3 categories: 1) Above 300 nmol α-ketobutyrate mg⁻¹ h⁻¹ 2) 250–300 nmol α-ketobutyrate mg⁻¹ h⁻¹ 3) below 250 nmole α-ketobutyrate mg⁻¹ h⁻¹ for *Pseudomonas* whereas it was 1) >120 nmol α-ketobutyrate mg⁻¹ h⁻¹ 2) 80-120 nmol α-ketobutyrate mg⁻¹ h⁻¹ 3) <80 α-ketobutyrate mg⁻¹ h⁻¹ for *Paenibacillus* isolates.

In the present study, among the twenty *Pseudomonas* isolates, 2 isolates *viz.*, PF-5 and PF-19, constituted the first category, 13 isolates *viz.*, PF-1, PF-2, PF-3, PF-6, PF-7, PF-8, PF-10, PF-12, PF-13, PF-14, PF-15, PF-16 and PF-18 constituted second category and the remaining isolates were ranked in the third category.

Among the twenty *Paenibacillus* isolates two isolates *viz.*, PB-5 and PB-19 constituted the first category, 13 isolates *viz.*, PB-1, PB-2, PB-3, PB-6, PB-7, PB-8, PB-10, PB-12, PB-13, PB-14, PB-15, PB-16 and PB-18 constituted second category and the remaining isolates were ranked in the third category.

The bacterial production of ACC-deaminase enzyme has been reported by many authors (Glick *et al.*, 1997; Jacobson *et al.*, 1994; Shah *et al.*, 1998). Sarvanakumar and Samiyappan (2007) reported the production of ACC-deaminase by *Pseudomonas fluorescens* as 342 nmol α-ketobutyrate mg⁻¹ h⁻¹. In
the present study, a maximum amount of ACC-deaminase (328 \( \alpha \)-ketobutyrate \( \text{mg}^{-1} \text{h}^{-1} \)) enzyme produced by the *Pseudomonas* isolate *viz.*., PF-5, an isolate collected from Kalararuthur, Ulundurpet taluk of Tamilnadu and the enzyme production by all the other *Pseudomonas* isolates were below this level. The other isolates showed the enzyme production in a range of 229 to 319 nmol \( \alpha \)-ketobutyrate \( \text{mg}^{-1} \text{h}^{-1} \). Among the different *Paenibacillus* isolates, the isolate PB-5, produced a maximum of ACC-deaminase (acd) enzyme *viz.*., 132 nmol \( \alpha \)-ketobutyrate \( \text{mg}^{-1} \text{h}^{-1} \). Ghosh *et al.* (2003) reported the ACC deaminase (acd) activity in three *Bacillus* sp. which stimulated the root elongation in *Brassica campestris*. The result of the present study also confirmed the production of ACC-deaminase by *Pseudomonas* and *Paenibacillus* isolates and in conformity with the earlier finding of Sarvanakumar and Samiyappan (2007) and Ghosh *et al.* (2003).

5.4. **Interstrain differences of *Pseudomonas* and *Paenibacillus* isolates for PGPR characterisation**

The two efficient ACC-deaminase producing *Pseudomonas* isolates *viz.*., PF-5 and PF-19 and two efficient ACC-deaminase producing *Paenibacillus isolates* *viz.*., PB-5 and PB-19 were screened further for the PGPR characteristics with respect to phosphate solubilizing efficiency (SE), Indole acetic acid (IAA), siderophore and exopolysaccharide (EPS) production under *in vitro* condition.

Interstrain differences for PGPR characteristics has been reported in *Pseudomonas* by many workers (Husen *et al.*, 2011; Sahroona *et al.*, 2006; Sarvanakumar and Samiyappan, 2007). The interstrain differences of
*Paenibacillus* isolates for PGPR characteristics has been reported by Von der Weid *et al.* (2000) and Faria da Mota *et al.* (2002).

### 5.4.1. IAA production by PGPR isolates

In the present study, all the four efficient isolates of *Pseudomonas* and *Paenibacillus* viz., PF-5, PF-19, PB-5 and PB-19, respectively were evaluated for their IAA (µg/mL) production efficiency. The IAA production by *Pseudomonas* isolates ranged from 5.0 to 5.7 µg/mL, whereas it was 4.7 to 5.3 µg/mL for *Paenibacillus* isolates. The maximum IAA production was found with isolate, PF-5 (*Pseudomonas*) and isolate PB-5 (*Paenibacillus*) followed by other isolates. Variation in the production of IAA by different isolates of *Pseudomonas* has been reported by Ahamed *et al.* (2005), Francesco *et al.* (1987), Khakipour *et al.* (2008), and Peyvandi *et al.* (2010). The interstrain difference of *Paenibacillus* on IAA production has been reported by Idris *et al.* (2007) and Klopper *et al.* (2004). The results of the present study are in conformity with the above earlier findings.

### 5.4.2. Siderophore production by PGPR isolates

The siderophore production of the two *Pseudomonas* isolates viz., PF-5 and PF-19 and two *Paenibacillus* isolates viz., PB-5 and PB-19 revealed the existing of interstrain difference among the PGPR isolates. The two *Pseudomonas* isolates produced salicylic acid, as a component of siderophore, in a range of 2.98 to 3.90 (µg/mL) whereas it was 2.65 to 3.07 (µg/mL) for *Paenibacillus* isolates.

The siderophore production by *Pseudomonas* sp. has been reported by many authors (Dhanya and Potty, 2007; Kloeppepler *et al.*, 1980; Sayeed *et al.*, 2005). Rachid and Ahamed (2005) reported the existence of interstrain
difference among the different *Pseudomonas fluorescens* isolates for siderophore production. The siderophore production by *Paenibacillus polymxa* SQR-21 has been reported by Raza and Schen (2010). In the present study also, all the *Pseudomonas* and *Paenibacillus* isolates produced siderophore under *in vitro* condition but with variation in their capability and the results of present study are in conformity with the above earlier findings.

**5.4.3. Phosphate solubilising efficiency of PGPR isolates**

In the present study, the two *Paenibacillus* isolates viz., PB-5 and PB-19 showed phosphate solubilization in PVK medium but with difference in their phosphate solubilizing efficiency (SE). The *Paenibacillus* isolate, PB-5 recorded a maximum ‘P’ solubilisation in PVK medium whereas the isolate, Viz., PB-19 exhibited the poor performance.

The phosphate solubilising efficiency of *Paenibacillus polymyxa* has been reported by many authors. El-Khomy (2004) studied the phosphate solubilising efficiency of *Paenibacillus polymyxa* in PVK medium and reported that the phosphate solubilisation in PVK medium increased after 2 days of incubation and then the solubilisation stopped although the inoculum was still growing. He reported a higher solubilisation of phosphate after 48h of incubation in the same medium. In the present study also, the isolate PB-5 recorded the phosphate solubilising efficiency as 180.5 µg/mL after 48 h incubation and in conformity with the findings of El-Khomy-2004.

**5.4.4. EPS production by PGPR isolates**

The exopolysaccharide (EPS) production potential of *Paenibacillus* isolates Viz., PB-5 and PB-19 revealed the existence of distinct differences between the two isolates as regards to exopolysaccharide (EPS) production. The
interstrain difference of *Paenibacillus* isolates for EPS production has been reported by many authors (Gjung-Kahng *et al.*, 2001, Haggag, 2007, Liu *et al.*, 2009 and Liu *et al.*, 2010). In the present study, all the *Paenibacillus* isolates were able to produce both water soluble and alkali stable EPS, but with variation in the production of the same. The *Paenibacillus* isolate *viz.*, PB-5 relatively recorded more EPS production than the isolate PB-19. The results of present study clearly envisaged the existence of interstrain difference for EPS production between *Paenibacillus* isolates and in conformity with the earlier findings.

### 5.5. Speciation of *Pseudomonas* and *Paenibacillus* isolates

On the basis of the performance for PGPR characteristics, one efficient isolate from each PGPR genera *viz.*, PF-5 from *Pseudomonas* and PB-5 from *Paenibacillus*, were selected and subjected to speciation of the same. The efficient *Pseudomonas* isolate *viz.*, PF-5 was compared for the parameters, as mentioned in Bergey’s manual of Systematic Bacteriology – 2\(^{nd}\) edition, for the speciation of *Pseudomonas* with other *Pseudomonas* species *viz.*, *Pseudomonas fluorescens* (ATCC 7283), *Pseudomonas putida* ATCC (12633), *Pseudomonas aureginosa* (ATCC 10145) and *Pseudomonas stutzeri* (ATCC 14405). In the same way, the efficient isolate of *Paenibacillus* *viz.*, PB-5, was compared for the parameters as mentioned by Claus and Berkeley (1986), regarding the speciation of *Paenibacillus* with other *Paenibacillus* species *viz.*, *Paenibacillus polymyxa* (ATCC 842), *Paenibacillus azotofixans* (ATCC 35681), *Paenibacillus macerans* (ATCC 8244), *Paenibacillus pabuli* (ATCC 43899) and *Paenibacillus amyloyticus* (ATCC 9995). Based on the results of the
study, the isolates *viz.*, PF-5 and PB-5 were confirmed as *Pseudomonas fluorescens* and *Paenibacillus polymyxa*, respectively.

5.6. Mechanism of coflocculation

Flocculation is a widespread phenomenon in the microbial world and occurring under certain physiological conditions (Calleja, 1984). Free living bacteria such as *Pseudomonas* and *Paenibacillus* are known for their capacity to flocs and this property positively affect the dispersal and survival of microbes in soil (Nikitina *et al*., 2001). Flocculation of microbial cells is the pre-requisite for encystations/sporulation which plays a key role in survival of microbes under stress conditions. Flocculation of various microorganisms has been reported by many workers (Deinema and Zevenhuizen, 1971; Madi and Henis, 1989; Mathysse *et al*., 1981; Sadasivan and Neyra, 1985). Crabtree *et al*. (1966) reported that flocculation augmented poly-β-hydroxybutyrate (PHB), storage granules, in microorganisms. The encystment and PHB production in *Azotobacter* sp. have been well documented. In *Azospirillum*, Lamm and Neyra, (1981) reported that ‘C’ forms were cysts which exhibited resistance to desiccation and temperature. Okon *et al*. (1976) and Tal and Okon, (1985) studied the role of PO$_4$ and C:N ratio on PHB production in *Azospirillum*. Bleakley *et al*, (1988) studied the cyst formation of *Azospirillum lipoferum* on hydroxybutyrate, as carbon source. Neyra *et al*. (1995), proposed the new generation of inoculants containing flocs of *Azospirillum* and *Rhizobium* for common bean plants. Burdman *et al*. (1998) studied the various physical and chemical factors which affect the flocculation in *Azospirillum brasilense*. The concept of interbacterial flocculation was proposed by Gibbons and Nygaard (1970) during the study of oral bacteria. They studied the intergeneric
coflocculation between *Streptococcus mutans* or *S. sanguis* when mixed with *Actinomyces viscosus* during dental plaque formation and described the mechanism as the recognition between surface molecules on two different bacterial cell types so that a formation of mixed-cell flocs. Later, Mc Intire et al. (1978) described the mechanism of coflocculation between *Actinomyces viscosus* T14V and *Streptococcus sanguis* 34. Now, the concept of intergeneric coflocculation among bacterial species has been well developed and established (Cassel and London, 1989; Malik and Kakil, 2003). Kolenbrander and Andersen (1986) studied the intergeneric coflocculation among oral bacteria and reported the independent cell-to-cell interaction in coflocculation.

Neyra et al. (1995) proposed the concept of “Intergeneric coflocs” of PGPR cells, as a new generation of bioinoculant. Later Nikitina et al, (2000) confirmed the “Intergeneric coflocculation” of *Pseudomonas* and *Bacillus* sp. and they explained the mechanism as EPS-mediated one. They are also reported the entrapment of rhizobacterial cells within EPS matrix produced by the coflocculation partners. Suh et al. (1999) reported the bioflocculation of *Bacillus* sp DP-152 and reported that the bioflocculant be an EPS, containing sugars like glucose, mannose, galactose and fructose at 8:4:2:1 ratio. In this laboratory, Kannan (2010) reported the EPS mediated intergeneric coflocculation of *Azospirillum* and *Paenibacillus*.

In the present study, the role of various factors viz., inoculum level, growth phase, culture media, temperature, pH level, effect of divalent cations and chelating agents, was studied on the “Intergeneric coflocculation” of *Pseudomonas* (PF-5) and *Paenibacillus* (PB-5) under *in vitro* condition.
The importance of inoculum levels of coflocculation partners in intergeneric coflocculation has been reported by Gibbons and Nygaard (1970), Kolenbrander and Andersen, (1982) and Kolenbrander, (1988). Gibbons and Nygaard (1970) reported the importance of equal number of coflocculation partners to obtain a visibly stronger coflocculation. The higher inoculum levels leads to the formation of rosette type colonies that are visible only with microscopes (Kolenbrander and Andersen, 1982). In the present study, it was observed that both the PGPR isolates Viz., *Pseudomonas* and *Paenibacillus* recorded the highest coflocculation percentage at a inoculum level of $10^7:10^7$ and any increasing or decreasing values to this level of inoculum caused a reduction in their coflocculation ability. The results of the present study clearly revealed the importance of inoculum level of PGPR partners to achieve the maximum coflocculation percentage and also in conformity with the earlier findings of Gibbons and Nygaard (1970), Kolenbrander and Andersen (1982) and Kolenbrander (1988).

The effect of different growth phases of the PGPR partners *viz.*, *Pseudomonas* and *Paenibacillus*, on the coflocculation percentage of PGPR cells was studied. Growth phase of the PGPR partners played a critical role on the coflocculation percentage of the same. Among the different growth phases tested, the stationary growth phase of coflocs partners recorded the highest coflocculation percentage followed by lag and log phases. The lowest coflocculation percentage was recorded with the log growth phase of PGPR partners and revealed the fact that active metabolic state of the microbial cell was not conducive for coflocculation. Kolenbrander *et al.* (1983) and Kolenbrander and Williams (1981) reported the effect of growth phases on the coflocculation of *Streptococcus* with other human oral bacterial isolates,
collected from the same site. The effect of the culture age on the composition of
the cell surface of bacteria has been reported by many workers (Burdman et al.,
2000a). Nikitina et al. (2000) reported that the coflocculation of *Azospirillum
brasileense* SP7 (S) and SP.7.2.3 changed with the culture age. The results of the
present study clearly revealed the determining role of growth phase of PGPR
cells on coflocculation percentage of the same and also in conformity with the
earlier findings.

The effect of different cultural conditions *viz.*, N-free and N-
supplementation, of PGPR isolates *Viz.*, *Pseudomonas* and *Paenibacillus* on
coflocculation percentage of the same was studied. Cultural conditions of the
PGPR partners played a key role in determining the coflocculation percentage
of the same. Between the two cultural conditions studied, namely, PGPR cells
grown in N-free medium and PGPR cells grown in N-supplemented medium,
the PGPR cells collected from N-free medium recorded more coflocculation
percentage than the PGPR cells collected from N-supplemented medium.
Sadasivan and Neyra (1999) reported the effect of high C : N ratio on the
coflocculation of *Azospirillum brasilense* Cd. strain. Kolenbrander (1988)
summarized the effect of culture medium on the coflocculation of
*Streptococcus* and *Actinomyces* suspensions, collected from human oral
ecosystem. Burdman *et al.* (1998) reported that when *Azospirillum brasilense*
isolates FAJ 0204 grown under high C : N ratio medium accumulated high
amounts of poly-β-hydroxybutyrate with a change in cell surface properties,
namely, a well-defined electron-dense layer outside the outer membrane. It has
been previously reported that the cells of *Azospirillum brasilense* growing at
high C:N ratio tends to flocs and it was also shown that the amount of arabinose
present in the EPS correlates with the extent of cell flocculation of different
Azospirillum isolates (Burdman et al. 2000b). The results of present study clearly revealed the highest coflocculation percentage of PGPR cells, collected from N-deficient medium, suggested the changes in cell surface characteristics of PGPR cells which resulted in more coflocculation percentage of the same.

The effect of different growth temperature viz., 25, 30, 35, 40 and 45, on the coflocculation percentage of PGPR cells viz., Pseudomonas and Paenibacillus, maintained in Co-AG buffer was studied. The growth temperature level of PGPR cells played a critical role in determining the coflocculation percentage of the PGPR partners. The increasing level of growth temperature showed an increasing trend in coflocculation percentage upto 35°C and thereby a reduction in the same was observed. Ketstrup and Funder-Nielsen (1974) reported the positive effect of growth temperature in determining the coflocculation of Streptococcus with Fusobacterium and Actinomyces. Burdman et al. (1998) reported the positive effect of growth temperature on coflocculation of Azospirillum brasilense cd. cells. They reported that Azospirillum grown under high C:N ratio recorded a higher flocculation at higher temperature level whereas the highest temperature beyond the optimum temperature level caused dispersion of the coflocs. The results of the present study are also in conformity with the above findings.

The effect of different levels of buffer pH on the coflocculation percentage of PGPR cells viz., Pseudomonas and Paenibacillus, was studied. Buffer pH exerted a positive role on the PGPR partners to attain maximum coflocculation percentage. Among the different buffer pH levels tested, 7.5 level of buffer pH, recorded the highest coflocculation percentage followed by 6.5, 6.0 and 7.0 buffer pH levels. Sadasivan and Neyra (1985) and Madi and
Henis (1989) reported the positive effect of pH on coflocculation of *Azosprillum* cells and added that there was dispersion of *Azospirillum* cell at neutral pH (pH 7.0) while any increase or decrease to this pH level augmented the coflocculation of *Azospirillum* cells. Burdman et al. (1998) reported the involvement of charged groups in this phenomenon and the *Azospirillum* isolates Cd and FAJ 0204 responded differentially to the levels of pH. They also added that the negative ionized groups of bacterial cell surface could be neutralized by protonation, thus reducing the strength of repulsive forces between bacteria and leading to coflocculation. The results of the present study also revealed the differential response of PGPR cells to different pH levels and in conformity with the above earlier findings.

The effect of addition of different divalent cations *viz.*, Ca$^{2+}$, Mg$^{2+}$ and Ba$^{2+}$ to CO-AG buffer on the coflocculation percentage of PGPR cells *viz.*, *Pseudomonas* and *Paenibacillus*, was studied. Addition of divalent cations to Co-AG buffer augmented the coflocculation percentage of PGPR cells, positively. Among the different divalent cations tested, the addition of Ca$^{2+}$ was found to augment the phenomenon to a higher level followed by Mg$^{2+}$ and Ba$^{2+}$. Jana (1999) reported the positive role of Ca$^{2+}$ ions in the augmentation of cell surface hydrophobicity in *Pseudomonas fluorescens*. Smit et al. (1992) reported that no flocculation occurred in the absence of Ca$^{2+}$ in *Saccharomyces cerevisiae*. Rose (1984) reported the positive role of divalent cations on the flocculating ability of *S. cerevisiae*. Mill (1964) reported that Ca$^{2+}$ ions acting as bridges in the coflocculation of yeast cells. Miki et al. (1982) emphasized the importance of Ca$^{2+}$ ions in yeast cell flocculation but in some cases magnesium and manganese ions may act as substitutes. The results of the
The present study clearly revealed the importance of Ca$^{2+}$ ions in augmenting the coflocculation between PGPR cells and in conformity with the above findings.

The effect of addition of chelating agents \textit{viz}., EDTA or EGTA to coflocculation buffer on the coflocculation percentage of PGPR cells \textit{viz}., \textit{Pseudomonas} and \textit{Paenibacillus}, was studied. Addition of chelating agents to Co-AG buffer reduced the coflocculation percentage of PGPR cells, significantly. Between the two chelating agents tested, the addition of EDTA to the Co-AG buffer reduced the coflocculation percentage of PGPR cells to a marked level followed by EGTA. Burdman \textit{et al.} (1998) reported the effect of EDTA and EGTA on the dispersion of \textit{Azospirillum} coflocs. They suggested the involvement of outer membrane proteins (OMP) of microbial cells in cell-to-cell adhesion. They also added that higher concentrations of these compounds drastically reduced the cell viability and caused partial lysis of bacteria. Madi and Henis (1989) showed the treatment with Na EDTA resulted in the dispersion of \textit{Azospirillum} coflocs while the addition of dialyzed EDTA restored their flocculation capacity and suggested the role surface-located proteins involved in the coflocculation process. The results of the present study also revealed the positive influence of EDTA in dispersing the PGPR coflocs and emphasized the role of surface located protein in coflocculation. These findings are in conformity with earlier findings of Madi and Henis (1989) and Burdman \textit{et al.} (1998) in \textit{Azospirillum} coflocculation.

The effect of addition of plant seed material \textit{viz}., \textit{Moringa oleifera}, \textit{Strychnos potatorum}, \textit{Sappindus emaginatus}, \textit{Allium cepa} and \textit{Asteracantha longifolia} on the induction of artificial flocculation among PGPR isolates \textit{viz}., \textit{Azospirillum} and \textit{Paenibacillus} under log phase of growth, was
studied. Among the different plant seed material tested, the seed material of *Moringa oleifera*, induced maximum coflocculation percentage among PGPR isolates than other seed materials. The flocculation ability of *Moringa* seed materials has been discussed by many authors (John, 1988; Ndabigenesere and Narasaiah, 1998; Okuda *et al.*, 2000; Broin *et al.*, 2002; Muyibi and Alfugara, 2003; Jayaraj *et al.*, 2004; Oluduro and Aderiye, 2007; Amagloh and Benang, 2009) and used the same primarily in water treatments.

Heller *et al.* (2000) emphasized the role *Moringa oleifera* seeds, as water clarifier and explained that water soluble proteins released from the crushed seed kernels functioned as natural flocculation agents. In the present study, the water soluble protein released from the seed kernel of *Moringa oleifera* might be the reason for the flocculation of PGPR cells. However, there were no earlier reports available on the artificial coflocculation of PGPR cells by using pant seed material and this is the first comprehensive study on the subject.

The thermal and desiccation tolerance of natural and artificial PGPR coflocs, of *Pseudomonas* and *Paenibacillus* were evaluated under *in vitro* condition. It was observed that the thermal and desiccation tolerance of PGPR coflocs, (natural), were found to be maximum when compared to PGPR coflocs (artificial). Increase in the content of poly-B-hydroxybutyrate (PHB), during flocculation, and their positive role on the enhancement of thermal and desiccation tolerance in *Azospirillum* has been reported by many authors (Bleakley *et al.* 1988; Sadasivan and Neyra, 1985). Olubayi *et al.* (1998) reported that the PHB content of flocculated *Azospirillum* cells ranged from 60-65 per cent of cell dry weight. The high spore count of the gram positive,
Paenibacillus polymyxa, cultured under high C : N ratio medium has been reported by Gordon, (1973). The increase in the thermal and desiccation tolerance of PGPR coflocs (natural) might be the result of the increase in the content of carbon reserve materials and the high spore count of the PGPR cells in the coflocs and the results of the present study are in conformity with the above findings.

The application effect of different formulations viz., vegetative cells of Pseudomonas and Paenibacillus coinoculation of Pseudomonas and Paenibacillus and natural and artificial coflocs of PGPR cells viz., Pseudomonas and Paenibacillus on the enhancement of seed vigour index of rice was studied. The phytostimulatory effect of Pseudomonas cells has been already reported (Ahamed et al., 2005; Glick et al., 2005). Neyra et al. (1999) described the phytostimulatory effect of “Intergeneric coflocs”, containing Azospirillum and Rhizobium cells, on the enhancement of growth parameters in faba bean. Moreover, the phytostimulatory effect of bacterial EPS has been demonstrated in Azospirillum (Burdman et al., 2000b). In the present study the application of EPS rich, PGPR coflocs, (natural) containing Pseudomonas and Paenibacillus cells augmented the seed vigour index of rice to a higher level than the application of PGPR coflocs (artificial) containing Pseudomonas and Paenibacillus cells and individual application of PGPR cells and emphasized the positive role EPS rich, PGPR coflocs (natural) on the augmentation of seed vigour index of rice and in conformity with the earlier findings of Neyra et al. (1999).

The application effect of different formulations viz., of Pseudomonas or Paenibacillus, coinoculation of Pseudomonas and Paenibacillus cells and
cophlocs application (natural and artificial) of *Pseudomonas* and *Paenibacillus* on the enhancement of rice root adhesion was studied. The adhesion mechanism of *Pseudomonas* cells has already been reported in many crop plants by many authors (O’ Sullivan and O’Hara, 1992; Wei et al., 1996).

Katupitiya et al. (1995), Levanony and Bashan (1989) and Michels et al. (1998) reported the poor adhesion of floc negative mutans of *Azospirillum* to wheat roots and emphasized the positive role of EPS in the adhesion processes. Sadasivan and Neyra (1985) reported the improved adhesiveness of *Azospirillum* biofloc with plant roots and suggested the positive role of EPS in the early events of adhesion to plant roots. The adhesion of EPS rich *Paenibacillus* cells to various plant roots has also been reported (Puente et al., 2009). The results of the present study clearly revealed the improved to rice root adhesion of EPS rich PGPR coflocs, (natural) when compared to PGPR coflocs (artificial) and individual application of PGPR cells and emphasized the positive role of EPS rich, natural cofloc of rhizobacterial isolates in the early events of adhesion to rice root.

The application effect of different formulations viz., vegetative cells of *Pseudomonas* or *Paenibacillus*, coinoculation of *Pseudomonas* and *Paenibacillus*, and natural and artificial coflocs of *Pseudomonas* and *Paenibacillus* on the incidence of *Xanthomonas oryzae* in rice was studied under *in vitro* condition. In the present study, the natural coflocs consisting isolates of *Pseudomonas* and *Paenibacillus* cells reduced the leaf blight disease incidence (*Xanthomonas oryzae*) to a higher level followed by the application of artificial coflocs consisting of *Pseudomonas* or *Paenibacillus*, coinoculation of *Pseudomonas* or *Paenibacillus* and individual application of
either *Pseudomonas* or *Paenibacillus*. Application of a mixture of 3 PGPR isolates *viz.*, *Bacillus pumilus* INR 7, *B. subtilis* GB 03 and *Curtobacterium flaccumfaciens* ME 1, as seed treatment, has resulted in more disease reduction when compared to isolates tested, singly. This might be due to different mechanisms of action of each PGPR strain against phytopathogens (Raupach and Kloepper, 1998). *Pseudomonas fluorescens* and *Burkholderia cepacia* have showed a synergistic effect on the suppression of rice sheath blight incited by *Rhizoctonia solani* (Sung and Chung, 1997). Similarly, the synergistic effect of PGPR strain mixtures on the biocontrol of phytopathogens has been reported frequently (Pierson and Weller, 1994). The results of the present study clearly revealed the efficient role of EPS-rich, PGPR coflocs (natural) on the reduction of bacterial leaf blight disease incidence. Kyunseok *et al.* (2008) reported the EPS application of *Burkholderia gladioli* and the elicitation of ISR in cucumber. Haggag (2007) reported the EPS application of *Paenibacillus polymyxa* in controlling the crown root disease of peanut. Pieterse *et al.* (2000) reported the positive role of ACC-deaminase containing rhizobacterial isolates on the modulation of ethylene in crop rhizosphere which leads to induction of systemic resistance (ISR) in *Arabidopsis thaliana*. Hence, the EPS rich natural coflocs, containing *Pseudomonas* and *Paenibacillus* cells on the reduction of leaf blight disease incidence suggested the synergistic effect of differential mode of action of rhizobacterial isolates *viz.*, EPS mediated ISR on the enhancement of ISR against *Xanthomonas oryzae* in rice. This is the first comprehensive report regarding the EPS rich PGPR coflocs (natural) application on the reduction of blast disease incidence in rice.
5.7. Role of different formulations of *Pseudomonas* and *Paenibacillus* cells on the enhancement of ISR mediated biocontrol of *Xanthomonas oryzae pv.oryzae* with special emphasis to biochemical and physiological aspects

PGPR are beneficial, free living, root colonizing bacteria exerting beneficial effects on host plant, including, plant growth promotion and biocontrol of phytopathogens. In recent years, the use of PGPR, as an elicitor of induced systemic resistance (ISR), in crop plants against different phytopathogens has been demonstrated under field conditions (Wei *et al*., 1996). PGPR induce resistance in plants against various fungal, bacterial and viral diseases (Liu *et al*., 1995), insect (Zehnder *et al*., 1997) and nematode pests (Oostendorp and Sikora, 1990; Sikora, 1992). Induction of systemic resistance by PGPR isolates against phytopathogens has been proved by spatially separating the pathogen and PGPR in the plants (van Peer *et al*., 1991).

PGPR brings about ISR through fortifying the physical and mechanical strengths of cell wall as well as changing the physiological and biochemical reaction of the host leading to a synthesis of defense chemicals against the challenge pathogen (Benhamou *et al*., 1996). Application of PGPR and induction of systemic resistance against plant pathogens has been reported by many workers (Anderson and Guerra, 1985; Benhamou *et al*., 1998; Hoffland *et al*. 1995; Maurhofer *et al*.,1994; M'Piga *et al*., 1997; van Peer *et al*. 1991; Zdor and Anderson, 1992). There are several bacterial determinants involved in the induction of systemic resistance by PGPR. The most important being the lipopolysaccharides (LPS) present in the outer membrane of bacterial cell, siderophore and salicylic acid production (van Loon *et al*., 1998). However,
there were only few reports regarding the use of rhizobacterial EPS, as bacterial determinant, on the elicitation of induced systemic resistance (ISR) in host plants (Guzzo et al., 2008), available. Kyungseok et al. (2008) reported the elicitation of ISR in cucumber plant against *Colletotrichum orbiculare* induced by the application of *Burkholderia gladioli* EPS. Haggag (2007) reported the positive effect of *Paenibacillus polymyxa* EPS on the elicitation of ISR against crown gall disease caused by *Aspergillus niger* in peanut. Raupauch and Kloeper (2000) suggested the use of mixture of PGPR isolates, having multiple mechanism of action for the maximization of ISR in crop plants.

In the present study, the effect of different formulations of *Pseudomonas* and *Paenibacillus* cells *viz.*, single strain inoculation of either *Pseudomonas* or *Paenibacillus*, co-inoculation of *Pseudomonas* and *Paenibacillus*, coflocs of *Pseudomonas* and *Paenibacillus* (natural), together with challenge inoculation of *Xanthomonas oryzae pv. oryzae* on the induction of systemic resistance (ISR) in rice plants was studied under *in vitro* condition with special emphasis to biochemical constituents *viz.*, changes in phenolic content, sugar and starch level, and physiological aspects *viz.*, changing level of peroxidase (PO) and polyphenol oxidase (PPO) in rice plant.

5.7.1. Changes in phenol metabolism

In higher plants, phenolic compounds comprise a wide variety of aromatic compounds, such as, aromatic amino acids, anthocyanins, leucoanthocyanins, anthoxanthins, hydrobenzoic acids, phenolic glycosides, sugar esters of hydroxycinnamic acids, coumarin derivatives, lignins, lignans and flavonoids. It has been shown in many experiments that a correlation should exist between the degree of resistance and phenol level of healthy plants.
Post-infectional accumulation of phenols in the host is considered to be one of the resistant reactions (Farkas and Kiraly, 1962; Goodman et al., 1967; Kuc, 1963; Ramasamy and Prasad, 1974; Ragunathan, 1972; Sakamoto, 1950). Many experimental data in pathophysiology supported the contention that the phenolic level is higher in diseased plants than in healthy ones. It has been frequently observed that phenol accumulation takes place in all infected plant tissues. If a comparison is made between resistant and susceptible combinations, i.e., incompatible and compatible host-pathogen pairs, it is apparent that a more rapid accumulation of phenolics takes place in the incompatible host-pathogen complex than in compatible ones.

The mechanism by which phenols confer resistance may be due to:

1. Direct toxicity of phenols to the pathogen.
2. The oxidation products of phenols, such as, quinines are more toxic to the pathogen.
3. Interference with electron transport system leading to a blockage of energy release.
5. Oxidized phenols are strong inhibitors of pectinolytic enzymes produced by the pathogens.
6. Sometimes toxins of high molecular weight as well as oxidation products of catechins and particularly those of leuco-anthocyanins were found to be powerful inhibitors of pectinolytic enzymes.
7. The phenolic inhibitors suppress the IAA oxidase in plants and thereby increasing the levels of IAA.

Of these possible reasons the first three can be grouped as direct effects and next four as indirect effects of phenols on the pathogens.

In the present study, the application of PGPR coflocs (natural), containing Pseudomonas and Paenibacillus cells, augmented the total and OD phenol content of rice plant to a maximum level when compared to control (without any bioinoculation), coinoculation and the individual application of Pseudomonas or Paenibacillus during the challenge inoculation of Xanthomonas oryzae pv.oryzae in rice plant. Several workers endeavoured to find out a correlation between increased levels of total phenol and OD phenol with resistance (Farkas and Kiraly, 1962). It is well known that OD phenols are the most active forms of phenols and their oxidation products are more toxic. The oxidation is mediated by the enzyme PPO and PO and the resulting quinines are effective inhibitors of SH group of enzymes which may be inhibiting to the pathogen (Goodman et al., 1967). Several associations have been reported between phenolics and the resistance of plants to pathogen (Panda and Khush, 1995). Phenolic acids are involved in phytoalexin accumulation, biosynthesis of lignin and formation of structural barriers which play a major role in resistance against phytopathogens. Oxidation of phenols to oxidized products (quinine) play an important role in induced systemic resistance (ISR) which limits the activities of fungal phytopathogen. (Hassan et al., 2007; Shalaby et al., 2001). Ragab et al. (2009) reported the increased phenolic content during the induction of systemic resistance against root rot of basil using some chemical inducers.
Usharani (2005) studied the application effect of *Pseudomonas fluorescens*, as PGPR, on the accumulation of total and OD phenol content of rice plant and reported that the increased accumulation of total and OD phenol content of rice plant during the PGPR application and challenge inoculation of *Pyricularia oryzae* might be the reason for the low incidence of the disease. Mishra *et al.* (2006) reported the *Rhizobium* mediated induction of phenolics in rice (*Oryza sativa* L.). However, there were no earlier reports regarding the application effect of PGPR coflocs (natural), containing *Pseudomonas* and *Paenibacillus* cells, on induction of phenolics accumulation in host plant, available.

**5.7.2. Changes in carbohydrate metabolism**

In the present study, the application of PGPR coflocs (natural), containing *Pseudomonas* and *Paenibacillus* cells, reduced the reducing and non-reducing sugar level in rice plants during the challenge inoculation of *Xanthomonas oryzae pv.oryzae* followed by PGPR coflocs (artificial) coinoculation and single strain inoculation of either *Pseudomonas* or *Paenibacillus* and control (without any bioinoculation).

As a major source of energy, the level of carbohydrates has great influence on the incidence and development of the disease. Tissues containing greater reserves of oxidisable carbohydrates are often more prone to pathogenic invasion than tissues containing low reserves. Altered carbohydrate metabolism of the host in response to infection was studied by several workers (Bhaskaran and Prasad, 1971; Jayasekhar, 1983; Kalyanasundaram, 1986; Kiraly and Farkas, 1959). The sugar content in healthy and pathogen inoculated plants
was very often correlated with the resistance mechanism (Horsfall and Dimond, 1957).

In the present study, the reducing sugars, and non-reducing sugars, were found to decrease with PGPR coflocs (natural) application, containing *Pseudomonas* and *Paenibacillus* cells together with challenge inoculation of *Xanthomonas oryzae pv.oryzae*. The bioinoculation caused reduction in the level of above sugars in rice plant. Maximum reduction was encountered at coflocs (natural) application, containing *Pseudomonas* and *Paenibacillus* cells.

The reduction in the soluble sugars may be due to higher polymerization of sugars (Chaboussou, 1972). It may be presumed that the reducing sugar levels may be inadequate for the colonization of the pathogen. Post-infectional decrease in the sugar reserve of the host plant has been recorded by several workers (Rao and Nayudu, 1979; Sridhar, 1970). The decrease in reducing sugars may be due to:

1. Oxidation to meet the energy requirements for various resistant reactions,

2. Polymerization of reducing sugars to starch, and

3. Utilization of the reducing sugars for the synthesis of phenolic compounds through shikimic acid pathway (Uritani and Stahman, 1961).

The reduction in non-reducing sugar content may be due to its conversion to reducing sugars to meet the above requirements. In the present study, the content of reducing and non-reducing sugars decrease with the PGPR coflocs (natural) application.
It may be assumed that the pronounced decrease in the reducing sugars in challenge inoculated plants may be due to the rapid conversion of the soluble sugars to phenols or starch. Thus, reduction of reducing sugars, as a result of coflocs (natural) application, containing *Pseudomonas* and *Paenibacillus* cells, causing shortage of readymade energy source, polymerization of sugars and accumulation of phenols. All these making an unfavourable situation for the pathogen to develop and establish in the host. The higher rate of reduction in the native level of reducing sugars and increase in starch content may be one among the vital phenomena, contributing resistance to the plant. The accumulation of starch in PGPR natural coflocs applied plants may also be due to stimulated activity of starch synthetase (Murata and Akazawa, 1969). Moreover the starch synthesising enzyme is protected from being inactivated by the application PGPR natural coflocs in rice plants (Evans and Wildes, 1971). The reasons for the accumulation of starch in resistant plants than in the susceptible one may be due to:

1. Low starch content and at the same time its greater utilization due to greater spread of the pathogen without PGPR natural coflocs application in rice plants, and

2. Greater accumulation of starch and lower utilization due to poor establishment of the pathogen with PGPR natural coflocs application in rice plants.

The results of the present study clearly revealed the positive effect of PGPR natural coflocs, containing *Pseudomonas* and *Paenibacillus* cells, on the elicitation of ISR, in rice plants during the challenge inoculation of *Xanthomonas oryzae pv.oryzae*. 
5.7.3. Changes in peroxidase (PO) and Polyphenol oxidase (PPO) content

In the present study, the application of PGPR natural coflocs, containing *Pseudomonas* and *Paenibacillus* cells, augmented the peroxidase (PO) and polyphenol oxidase (PPO) content to a higher level in rice plants during the challenge inoculation of *Xanthomonas oryzae pv. oryzae* when compared to control (without bioinoculation). The PGPR natural coflocs containing *Pseudomonas* and *Paenibacillus* cells, recorded the highest performance for these parameters than other formulations *Viz.*, coinoculation and single strain inoculation of PGPR cells.

5.7.4. Physiological changes

Peroxidase (PO) and polyphenol oxidase (PPO) are the two key enzymes for the oxidation of phenolic compounds in plants and the resulting quinones were effective inhibitors of SH-group of enzymes (Goodman *et al.*, 1967) which might be inhibiting to the phytopathogens. Peroxidase is an oxido-reductive enzyme that involve in wall binding process. Peroxidase also catalyzes the condensation of phenolic compounds into lignin and is associated with disease resistance in plants (Hammerschmidt *et al.*, 1995). The increase in peroxidase activity has an important function in secondary wall biosynthesis by polymerizing hydroxyl and methylhydroxycinnamic alcohols into lignin and forming rigid cross-linking between cellulose, pectin, hydroxy proline and lignin (Grisebach, 1981). Polyphenol oxidase (PPO) is a widespread enzyme found in plant cells. This enzyme dehydrogenating ortho-dihydroxy phenols to produce ortho-quinines. It indicates the higher activity towards hydroxylation of monophenol to diphenol. Ramamoorthy *et al.* (2002) reported that the *Pseudomonas fluorescens* inoculation induced the activities of PO and PPO in
tomato and hot pepper plants against *Pythium aphanidermatum*. In bean, induction of PO activity has been recorded due to the colonization of soil bacterium (Zdor and Anderson, 1992). Higher PO activity was noticed in cucumber roots treated with *Pseudomonas corrugata* and challenge with *Pythium aphanidermatum* (Chen *et al.*, 2000). The PAGE study indicated the PO and PPO isoforms were predominately expressed with *Pseudomonas fluorescens* treated roots in response to infection by the phytopathogen (Thipyapong and Steffens, 1997).

Sridhar *et al.* (1969) reported the increased activity of peroxidase on pathogen infection might be required for an additional deposition of lignin around the lesions induced by pathogen. Robb *et al.* (1964) described the increasing PPO activity and explained that it might be due to either solubilization of polyphenol from cellular compartments or activations of latent polyphenol activation.

Nandakumar (1998) reported that ISR has been correlated with two-fold increase in peroxidase activity. He also stated that two peroxidase isoforms (35 KDa) have been induced in PGPR treated plants challenge inoculated with rice sheath blight pathogen (*Rhizoctonia solani*). Similarly, in sugarcane PGPR mediated ISR against *Colletotrichum falctum* enhanced the peroxidase level in host plants (Viswanathan and Samiyappan, 1999).

The results of the present study clearly envisaged the positive role of PGPR natural coflocs, containing *Pseudomonas* and *Paenibacillus* cells, in augmenting the ISR against *Xanthomonas oryzae pv.oryzae* in rice plants. However, the mechanism of EPS rich, PGPR natural coflocs mediated ISR
Discussion

against *Xanthomonas oryzae pv.oryzae* in rice plant is still unclear and the subject needs further elaborate research.

5.8. Studies on the effect of different formulations of PGPR cells on the enhancement of growth and yield parameters in rice (*Oryza sativa* L.)

The effect of different formulations *viz.*, single strain inoculation, co-inoculation and cofloc application of PGPR cells *viz.*, *Pseudomonas* and *Paenibacillus* cells, together with 75 per cent of recommended dose of ‘P’ fertilizers, on various growth parameters *viz.*, height, dry weight, organic carbon content, ‘P’ content of plant, IAA and chlorophyll content, and yield parameters *viz.*, grain and straw yield, was studied.

The effect of *Pseudomonas* inoculation in augmenting the growth parameters of rice has been studied by many authors (Mew and Rosales, 1986; Pierterse *et al.*, 2000; Ramezobpour *et al.*, 2010; Ramezobpour *et al.*, 2011; Saveetha *et al.*, 2009). The effect of *Paenibacillus* inoculation in augmenting the growth and yield parameters of rice has already been reported (Chanway and Holl, 1991; Dianese *et al.*, 1994; Guemouri-Athmani *et al.*, 2000; Heulin *et al.*, 1994; Holl *et al.*, 1988; Lindberg and Granhall, 1986; Mavingui *et al.*, 1994; Von der Weid *et al.*, 2000). The co-inoculation effect of *Azosprillium* and *Bacillus* sp. has also been reported by El-Komy (2005), Kandiannan *et al.* (2000) and Neelima *et al.* (2001).

Neyra *et al.* (1999) reported the positive effect of *Azospirillum* and *Rhizobium* coflocs application in augmenting the growth and yield parameters of faba bean. Many studies indicated that a level of 10-50 per cent ‘P’ could be saved due to the bio-inoculation of PGPR cells (Acharya *et al.*, 1999; Gunarto *et al.*, 1999). The aim of the present study is to evaluate the efficiency of
different formulations of PGPR cells in augmenting the growth and yield parameters of rice cv. BPT-5204 at 75 per cent recommended P level with a view to save 25 per cent of P fertilizers through bioinoculation.

In the present study, single strain inoculation of either *Pseudomonas* or *Paenibacillus* could augment the growth and yield parameters of rice to a higher level at 75 per cent recommended ‘P’ level when compared to control (100 per cent recommended ‘P’ level without bioinoculation). But, the effect was more pronounced when the two PGPR isolates *viz.*., *Pseudomonas* and *Paenibacillus* were co-inoculated at 75 per cent recommended ‘P’ level.

However, the effect was found to be the highest when the PGPR isolates were applied as natural coflocs. Regarding the single cell application, the application of *Pseudomonas* was found to enhance the growth and yield parameters of rice to a higher level than *Paenibacillus* inoculation at 75 per cent recommended ‘P’ level. Interestingly, the co-inoculation of *Pseudomonas* and *Paenibacillus* cells recorded the highest response than single cell application but not with natural cofloc application at 75 per cent recommended ‘P’ level. However, the response was found to be the maximum when the PGPR cells were applied as coflocs at 75 per cent recommended ‘P’ level. Greater plant height of rice due to the inoculation of *Pseudomonas* and *Paenibacillus* has been reported by many authors (Agarwal and Singh, 2000; Ramezobpour et al., 2011; Saveetha et al., 2009).

Increase in dry matter production of rice with *Pseudomonas* and *Paenibacillus* inoculation has been reported by Gnanamanikam and Mew (1992), Kaler et al. (2002), Pieterse et al. (2000), and Saveetha et al. (2009). The increase in the total ‘P’ content of rice due to the inoculation of
Pseudomonas and Paenibacillus has already been reported (Ding et al., 2005; Faria da Mota et al., 2002). The increase in chlorophyll content of rice leaves has been reported by Hegazi et al. (1985) and Kapulnik et al. (1985). The augmentation of grain and straw yield due to Pseudomonas and Paenibacillus inoculation has been reported by Ramezonpour et al. (2010) and Selvakumari et al. (2000). The co-inoculation effect of Pseudomonas and Paenibacillus on the enhancement of growth and yield parameters of cereals has been reported by many workers (Egamberdiyeva, 2000; El-Komy et al., 2004; Gholami et al., 2009; Nadeem et al., 2006).

The increase in the growth and yield parameters of common bean (Phaseolus vulgaris) due to Azospirillum + Rhizobium “Intergeneric coflocs” application is the only report, available on the subject (Neyra et al., 1999). However, the application effect of EPS rich, PGPR natural coflocs, containing bacterial genera of Pseudomonas and Paenibacillus, on the enhancement of growth and yield parameters of lowland rice crop has not been followed and reported in anywhere, so far.

In the present study, it was generally observed that “Intergeneric PGPR coflocs” consisting of Pseudomonas and Paenibacillus cells, augmented the growth parameters viz., height, dry weight, ‘P’ content of plant, IAA production in rice roots and yield parameters viz., grain and straw yield of rice to a higher level followed by co-inoculation of PGPR cells and single cell application of PGPR cells at 75 per cent recommended ‘P’ level.

It was concluded that “Intergeneric PGPR coflocs”, consisting of Pseudomonas and Paenibacillus cells, application together with 75 per cent recommended ‘P’ level could augment the growth and yield parameters of rice
when compared to the rice crop grown in 100 per cent recommended ‘P’ level without any bioinoculation and thus a saving of 25 per cent recommended ‘P’ levels could be possible through the application of “Intergeneric PGPR natural coflocs” in lowland rice. Moreover, the “Intergeneric PGPR natural coflocs application” augmented the ISR mediated biocontrol of bacterial leaf blight disease Viz., Xanthomonas oryzae pv. oryzae, a destructive fungal disease of rice crop and thus reduced the biological and environmental hazards and improved the crop productivity of lowland rice crop.