Antagonistic fluorescent pseudomonad bacteria that are target-specific, biodegradable, environment-friendly and most importantly, capable of colonizing in the sprayed environment are in the forefront for the effective control of plant pathogens. This group of bacteria is often predominate among plant associated rhizobacteria and is classified into two different groups such as strains that have the capability of synthesizing phytohormones and strains that have the ability to suppress the growth of phytopathogens (Bashan and Holguin, 1998). The role of fluorescent pseudomonads in agriculture has been a subject of interest due to their diversity and functional potential.

Beneficial fluorescent pseudomonads use one or more direct or indirect mechanisms that mediate soil fertility, plant health and plant protection. They are capable of improving plant nutrients uptake, tolerance to stress, salinity, metal toxicity and degradation of pesticide in soil (Crowley et al. 1996; Jacobsen, 1997; Andersen et al. 2001). This group of bacteria exhibits multiple functional traits such as solubilizing of inorganic phosphate (Katznelson and Bose, 1959) and iron (Neilands, 1984), production of vitamins (Rovira and Harris, 1961), phytohormones (Patten and Glick, 2002) and antimicrobial metabolites (Dwivedi and Johri, 2003; Raaijmakers et al. 2002). All these functional traits of fluorescent pseudomonads have been correlated for biological control of fungal pathogens and enhancement of growth and yield of different agricultural crops such as canola, tomato, soybean, wheat and rice (Abbass
and Okon 1993; Cattelan et al. 1999; de Fritas et al. 1997; Sakthivel and Gnanamanickam, 1987).

In the present investigation, three new broad-spectrum antagonistic fluorescent pseudomonad strains FP10, PUP6 and PUW5 have been isolated from rice rhizospheric soil of rice which is the important staple food crop of the world. On the basis of morphological, biochemical and 16S rRNA gene sequence similarity and subsequent molecular phylogeny analysis, strains FP10 and PUP6 were identified as *Pseudomonas aeruginosa* and strain PUW5 was taxonomically affiliated as *P. putida*.

Biolog system has been used by several investigators to study the carbon assimilation profiles (Fray et al. 1997). In this investigation, similar indigenous system (Hi-carbohydrate™ kits) has been used to study the carbon utilization profiles and phenotypic relatedness. Strains FP10, PUP6 and PUW5 utilized several carbon sources as identified by Hi-carbohydrate™ kit tests. Utilization of variety of carbon sources by fluorescent pseudomonads may play an important role in wide competition towards other microorganisms and therefore, will lead for adaptation to a variety of crop plants and soil types (Lungtenberg and Deckers, 1999). As indicated by other investigators (Palleroni 1984; Schroth et al. 1992), the bacterial colonization which is a pre-requisite for biocontrol may be related to their ability to utilize the root exudates of different plants. Therefore, ability to utilize specific organic substrates may be considered as one of the important traits involved in the selection of fluorescent pseudomonads as biological control agents.
Plant growth is often limited by insufficient phosphate availability in agricultural field soils. The low solubility of common phosphates such as $\text{Ca}_3(\text{PO}_4)_2$ hydroxyapatite and aluminum phosphate cause low phosphate availability. Regular application of chemical pesticides results in poor solubility of chemical fertilizers and therefore, agricultural soil posses considerable accumulation of insoluble phosphorus (Rodriguez and Fraga, 1999). Fluorescent pseudomonads have the ability to solubilize the insoluble phosphate and promote plant growth by converting the insoluble phosphate to soluble phosphate (Katznelson and Bose, 1959). Pseudomonad strains such as $P. \text{fluorescens}$ NJ-101 (Bano and Musarrat, 2004), $P. \text{fluorescens}$ EM85 (Dey et al. 2004), $P. \text{fluorescens}$ (Dalla, 1989), $P. \text{chlororaphis}$, $P. \text{savastanoi}$, $P. \text{pickettii}$ (Cattelan et al. 1999), and $P. \text{corrugata}$ (Pandey and Palani, 1998) have been reported as phosphate solubilizers. Therefore, phosphate-solubilizing strains having root colonization capability may influence the availability of phosphate to plant roots that is essential for the promotion of plant growth and enhancement of crop yield (Illumer and Schinner, 1995; Richardson et al. 2001). In an earlier study, $P. \text{fluorescens}$ strain NJ-101 isolated from agricultural soil was reported to release 74.6 µg/ml soluble phosphates from inorganic phosphate (Bano and Musarrat, 2004). In the present study, strains have been shown to release up to 76.33 µg/ml soluble phosphate.

Production of antimicrobial metabolites and organic acids is essential to decrease soil pH, which plays a major role in solubilization of phosphates and other nutrients. It is believed that microbial solubilization of insoluble phosphates in soil is through the release of organic acids (Gyaneshwar et al. 1998; Carrillo et al. 2002; Rodriguez et al. 2004). In the present investigation, significant decline in the pH of
the culture medium by strains FP10, PUP6 and PUW5 was observed during mineral phosphate solubilization, which suggested the microbial production of organic acids (Chen et al. 2006). However, in addition to acid production, other mechanisms can also cause phosphate solubilization (Nautiyal et al. 1990). Although phosphate solubilization is not necessarily correlated with acidity, from the data generated in this study a relationship could be ascertained between the acidity of medium and the release of soluble phosphates. Strains reported in this study may solubilize insoluble compounds due to the excretion of organic acids.

It is reported that the ACC deaminase producing strains increase root elongation and seed germination by lowering plant ethylene levels (Glick et al. 1995; Belimov et al. 2001). ACC deaminase producing fluorescent pseudomonads of pea and Indian mustard have been reported (Belimov et al. 2001). Therefore, ACC deaminase positive strain PUW5 reported in this study may play a vital role in root elongation and seed germination as reported earlier (Belimov et al. 2001). The phytohormone, IAA has been implicated to have dual role in influencing plant growth and in biocontrol of phytopathogens. In defense-related plant reactions IAA together with glutathione-s-transferases inhibits the germination of spore and growth of mycelium of different pathogenic fungi (Brown and Hamilton, 1993; Strittmatter, 1994). Microbial IAA production has been identified as an important criterion of promoting plant growth and development (Patten and Glick, 2002). Recently, Ramesh Kumar et al. (2005) reported the diversity of fluorescent pseudomonads associated with sugarcane and rice rhizospheric soils and observed the prevalent nature of IAA production in the strains inhabiting sugarcane rhizosphere. Martinez Noel et al. (2001) showed that the supply of IAA supply to excised potato leaves reduced the severity of
the disease incited by *Phytophthora infestans* (Martinez Noel et al. 2001). IAA has also been reported for increased plant growth of canola, tomato and wheat (Abbas and Okon, 1993).

In the present investigation, substantial production of IAA by strains FP10 and PUP6 was observed during stationary phase. Our observations are in good agreement with earlier reports suggesting the induction of IAA production in stationary phase of culture, probably owing to induction of key enzymes involved in IAA biosynthesis (Oberhansli et al. 1990; Patten and Glick 2002). Production of IAA, siderophore and phosphatase enzyme clearly suggests the plant growth promoting potential of strains FP10 and PUP6. Several studies indicated that AHL signal molecules serve not only as population density sensors but also for communication between cells of different species colonizing the plant rhizosphere. AHL molecule may be important for coordinating the various functions such as production of secondary metabolites as well as antibiotics of the fluorescent pseudomonad populations within the rhizosphere (Bassler 1999; Fray et al. 1999; Steidle et al. 2001). In the present investigation, strain FP10 tested positive for the production of AHL.

Strains reported in this study produced hydroxamate type of siderophore as evidenced on FeCl₃ amended CAS agar medium. The role of siderophores in control of diseases has been well documented (Baker and Sneh, 1986). It has been reported that siderophores produced by fluorescent pseudomonads help plants to overcome tropic stress from environment such as iron deficiency (Neilands 1984). Ferric ion has been shown to be more available to plants when it is composed of siderophores (Neilands, 1984).
Strains FP10, PUP6 and PUW5 produced fungal cell wall-degrading enzymes such as protease and chitinase. These enzymes are known to be involved in antagonistic activity against phytopathogenic fungi and insects (Chernin et al. 1995; Dunn et al. 1997). Selective microbial producers of chitinase are also reported to be efficient phosphate solubilizers (Krishnaraj and Goldstein, 2001). The absence of plant growth affecting traits such as production of HCN, cellulase and pectinase is an added advantage of rhizobacterial strains reported in this study.

Petroleum compounds are considered to be recalcitrant to microbial degradation and persist in ecosystems because they are less volatile and hydrophobic in nature, and thus, they pose a significant threat to the environment (Abed et al. 2004). The \textit{n}-paraffin members of petroleum or kerosene type hydrocarbons have been found as major contaminants in soil, water and bioaccumulation in mussels, invertebrates, fish and algae (Maidack et al. 1997; Tarkpea and Svanberg, 1982). In recent years, considerable efforts have focused on the screening of microorganisms with potential to degrade these pollutants (Li et al. 2000). Pseudomonads are known for their catabolic potential and their inherent capacity for degradation of recalcitrant xenobiotics (Ajithkumar et al. 2003; Gibson and Subramanian, 1984). Strain PUP6 reported in this study, for the first time, showed biodegradation of petroleum hydrocarbons, \textit{n}-alkane members that include short-chain (\textit{n}-dodecane), medium-chain (\textit{n}-hexadecane and \textit{n}-octadecane), and long-chain (\textit{n}-octacosane) hydrocarbons, petroleum fractions such as crude oil, and lubrication oil containing components of higher carbons (C >29). The use of such plant rhizosphere associated bacteria in the bioremediation of pollutants in soils has been proposed as an efficient way to spread degrading bacteria in contaminated soils (Andersen et al. 2001).
The production of antibiotics by fluorescent pseudomonads plays a vital role in suppression of phytopathogens. Antifungal metabolite, phenazine-1-carboxamide (PCN) production had already been identified in species of fluorescent pseudomonads such as *P. chlororaphis* (Chin-A-woeng et al. 1998) and *P. aeruginosa* (Mavrodi et al. 2001), and it was reported that biocontrol potential of PCN was at least 10 times greater than that of the other closely related antibiotic, phenazine-1-carboxylic acid (PCA). The 2,4-diacetylphloroglucinol (DAPG) has been well documented for its ability to suppress soil-borne fungal pathogens of root and seedling diseases. The DAPG producing strains such as *P. fluorescens* CHAO, F113, Q2-87 and Q8r1-96 have been successfully used against black root rot of tobacco, crown and root rot of tomato, *Pythium* damping-off of cucumber and sugar beet, take-all of wheat and cyst nematode and soft rot of potato (Vincent et al. 1991; Fenton et al. 1992; Keel et al. 1992; Harrison et al. 1993; Cronin et al. 1997; Duffy and De´fago 1997; Raaijmakers and Weller 1998).

Strains FP10 and PUP6 exhibited the presence of antibiotic genes encoding for DAPG and PCN respectively, when their genomic DNA was used as template in gene-specific PCR assays. However, strain PUW5 did not show the amplification of any antibiotic genes possibly due to the sequence variability or due to the involvement of new derivative of known or unknown antibiotics that mediate antagonism. Antifungal metabolites produced by strains FP10, PUP6 and PUW5 have been extracted and purified through chromatographic techniques and identified as 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxamide (PCN) and 5-methyl phenazine-1-carboxylic acid (MPCB), respectively on the basis of UV-Visible,
Infrared, NMR and mass spectroscopic analyses. These antifungal metabolites exhibited growth-suppression of phytopathogenic fungi.

Thomashow et al. (1990) demonstrated bioanalytical techniques such as TLC and HPLC to detect and quantify a variety of antibiotics including PCA, herbicolin A (Kempf et al. 1993), PRN (Kempf et al. 1994), gliotoxin (Lumsden et al. 1992) and DAPG (Keel et al. 1992; Bonsall et al. 1997). In the literature, among Fusarium-wilt pathogens, only *F. oxysporum* f. sp. *cepae*, the seedling pathogen of onion, *F. oxysporum* f. sp. *vasinfectum*, the seedling pathogen of cotton (Howell and Stipanovic 1980) and *F. graminearum*, the Fusarium-head blight pathogen of wheat have been reported to be inhibited by DAPG. In the present investigation, for the first time, the antifungal activity of DAPG against important banana pathogens, *F. oxysporum* f. sp. *cubense* and *Cylindrocladium* species such as *C. floridanum*, *C. scoparium* and *C. spathiphylli* has been reported. Microbial production of antibiotics, DAPG (0.5 to 3 mg/ml), PLT (1.5 to 2 µg/ml) and PRN (0.54 mg/ml) by biofertilizing and biocontrol strains have been reported in earlier studies (Broadhagen et al. 2005; El-Banna and Winkelmann, 1998; Haas and Keel, 2003). In the present study, the production of DAPG (14.5 µg/ml), PCN (1.05 µg/ml) and MPCB (6.0 µg/ml) by fluorescent pseudomonad strains has been reported. Strain efficiency and variations in the fermentation conditions often result in an alteration in antibiotic production. Considering the quantity of antibiotics by plant growth promoting and biocontrol strains of fluorescent pseudomonads reported by other investigators (Broadhagen et al. 2005; El-Banna and Winkelmann, 1998; Haas and Keel, 2003), strains FP10, PUP6 and PUW5 reported in this study may be considered as non-pathogenic but beneficial to plants and antagonistic against phytopathogenic fungi.
Due to their ability to produce antifungal metabolites such as PCN, DAPG and MPCB and fungal cell wall-degrading enzymes such as protease and chitinase, strains reported in the present investigation showed a broad-spectrum antifungal activity against major pathogens that attack important crops such as rice, banana, cotton, groundnut, tobacco, chili, sugarcane, mango and tea. Strains also showed innate biofertilizing traits such as production of phosphate-solubilizing enzymes, phytohormones, ACC deaminase, and AHL as well as bioremediation potential of hydrocarbons and agricultural pesticides. Therefore, strains FP10, PUP6 and PUW5 reported in the present investigation may be used as inoculants for plant growth promotion, disease control and bioremediation of soil pollutants for sustainable agriculture.