ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Isolation of *Pseudomonas fluorescens* species from rhizospheric soil of faba bean and assessment of their siderophores production

Fekadu Alemu

Department of Biology, College of Natural and Computational Sciences, Dilla University, P.O.Box. 419, Dilla, Ethiopia.

Manuscript Info

Manuscript History:

Received: 11 September 2013
Final Accepted: 22 September 2013
Published Online: October 2013

Key words:
Iron, King's B medium, *Pseudomonas fluorescens*, Rhizospheric soil, Siderophores

Abstract

Crop rhizosphere area were consisting certain beneficial microbes that may great role for crop and the environments. The present study was to isolate some of *Pseudomonas fluorescens* isolates possess a variety of promising properties which make them as better microbes. Twelve *Pseudomonas fluorescens* isolates were isolated on King's B medium from rhizospheric soil of faba bean and assessed their production of siderophores. The results indicated that all isolates tested have a potential to produce siderophores molecule. This molecule has a potential to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cell. So it could be concluded that all isolate can be produced siderophores that have antimicrobial activity on other microbes since these microbes can be use as biocontrol and also as Iron chelating for collecting of Iron. May also used for purfication of Iron from ores during mining.

Introduction

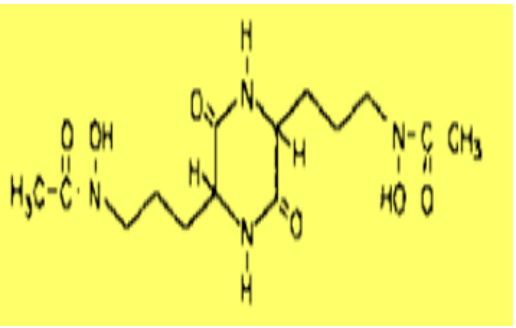
Pseudomonas fluorescens is a gram-negative, rod-shaped, and non pathogenic bacterium that is known to inhabit primarily the soil, plants, and water (Peix et al., 2009). It derives its name from its ability to produce fluorescent pigments under iron limiting conditions (Baysse et al., 2003). These bacteria belong to soil microorganisms that develop one of the very important soil process denitrification. Biological control is a promising approach for management of plant diseases (Anderson et al., 2004). *Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots (Kiely et al., 2006) and this applies also to the particular case of biocontrol agents this due to their ability to produce antimicrobial compounds (Haas and De´fago, 2005).

A Siderophore (Greek for iron carrier) is a low molecular weight (500-1000daltons), high affinity ferric iron-chelating compound secreted by organisms (Pal and Gokarn, 2010). Turfreijer (1942) proposed the term 'pyoverdine' for the yellow-green, fluorescent, water-soluble pigment of *P. fluorescens*. *Pseudomonas fluorescens* is one of the fluorescent pseudomonads that secrete pyoverdins (Meyer, 2000) for its essential requirement for iron. Pyoverdin is a yellow-greenish fluorescent siderophore involved in high affinity transport of iron into the cell (Budzikiewicz, 1992). Iron is the most important micronutrient used by bacteria and is essential for their metabolism, being required as a cofactor for a large number of enzymes and iron-containing proteins (Leong and Expert, 1990 and Neilands,1974).

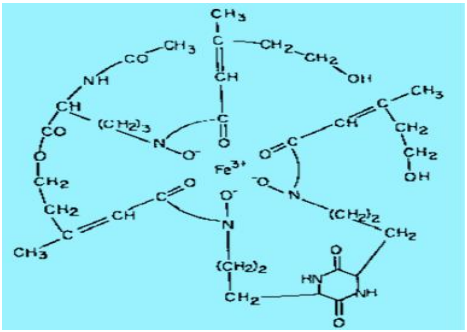
Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Indiragandhi et al., 2008). Some plant growth promoting rhizobacteria strains produce siderophores that bind Fe^{3+}, making it less available to certain members of native microflora (Kloepper et al., 1980a). Siderophores chelates iron and other metals contribute to disease suppression by conferring a competitive advantage to biocontrol agents for the limited supply of essential trace minerals in natural habitats (Hofte et al., 1992, Loper and Henkels, 1997). Duhme et al. (1998) have also demonstrated that catecholate siderophores have been suggested to participate in molybdenum acquisition. Siderophore-producing bacteria promote plant growth indirectly by sequestering the

limited iron in the rhizosphere, especially in neutral and alkaline soils, and thereby reduce its availability for the growth of pathogen (Alexander and Zuberer, 1991 and Subba Rao, 1999).

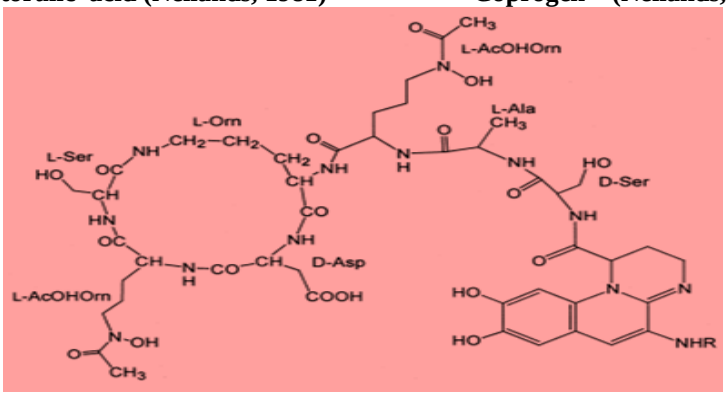
Siderophores are usually classified by the ligands used to chelate the ferric iron. The major groups of siderophores include the catecholates (phenolates), hydroxamates and carboxylates (Saharan and Nehra, 2011). Partial determination of its structure has shown that an unusual amino acid, 6-N-hydroxyornithine, is present in a cyclic peptide chain (Meyer, 1977). This amino acid is also a constituent of several hydroxamate iron-binding compounds: ferrichrome A (Zalkin et al., 1966), rhodotorulic acid (Atkin and Neilands, 1968), coprogen (Keller-Schierlein and Diekmann, 1970), ferribactin (Maurer et al., 1968). These siderophores facilitate iron transport into microorganisms (Neilands, 1974).



Rhodotorulic acid (Neilands, 1981)



Coprogen (Neilands, 1981)



Pyoverdins (Ferna'ndeza et al., 2003)

Figure 1. Type of siderophores

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria (Villacieros et al., 2003) known as plant growth promoting rhizobacteria (PGPR) (Kloepper et al.,1980b). Biocontrol strains have noticeably been observed at the root surface, (i.e. the rhizoplane) often forming microcolonies or discontinued biofilms in the grooves between epidermal cells (Couillerot et al., 2009).

This study will be provided comprehensive information on screen of microbes that have a great tendency to produce siderophores molecule that may be able to be used for collection of iron at purification during mining activites by appropriate application of this bacterium. Microorganisms require iron for a variety of metabolic processes, so they synthesize and secrete small organic molecules called siderophores that actively chelate iron. Therefore this study was designed to isolate certain *Pseudomonas fluorescens* species from faba bean rhizospheric soil and assessed their production of siderophores molecule.

MATERIALS AND METHODS

Soil sample collection

The rhizospheric soil samples were collected in an envelope from fields growing faba bean (*Vicia faba* L.) from five Kebales of Salele zone: Mechale wartsu at altitude of 2560 meters above sea level, Wachale at altitude of 2540 meters above sea level, Gore kateme at altitude of 2590 meters above sea level, Eveno at altitude of 2510 meters

above sea level and Gago at altitude of 2520 meters above sea level of North Showa of Oromiya Region of Salele zone, Ethiopia as indicated fig 2. The soils were brought to Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College Natural Sciences, Addis Ababa University.

Figure 2. Soil samples

Isolation of Pseudomonas fluorescens

Isolation of *Pseudomonas fluorescens* isolates studies were carried out on King's B medium (King et al., 1954). 1g of rhizosphere soil sample was suspended in 99 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spreaded on King's B medium plates. After incubation at 28^{o}C for 48 h the plates were exposed to UV light at 365 nm for few seconds and the colonies exhibiting the fluorescence were picked up and streaked on to the slants for maintenance, purified on King's B medium plates and also desigented as P f1-12 which stands for *Pseudomonas fluorescens* isolates used for further studies.

Assay for Siderophore Production

Siderophore production was tested by growing *Pseudomonas fluorescens* isolates on the king's B medium at 28^{o}c for 48 hours. The plates were exposed to UV light for few seconds and the colonies exhibiting the fluorescence (Ramyasmruthi et al., 2012).

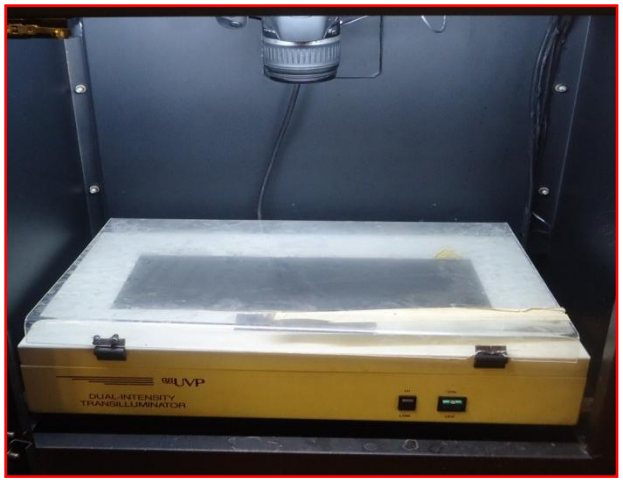


Figure 3. UV lamp apparatus set up

Data analysis

Date was analysis through qualitative description.

RESULTS AND DISCUSSION

During this research study, 12 *Pseudomonas fluorescens* were isolated from rhizospheric soil of healthy faba bean from five Kebales of Salale zone of Oromiya Region on King's B medium and observed under UV light at 365 nm for few seconds as shown in fig.4. Then it was purified again on same medium and observed under UV light. All the rhizospheric isolates were named as *Pseudomonas fluorescens* isolate 1=P f1, *Pseudomonas fluorescens* isolate 2=P f2, *Pseudomonas fluorescens* isolate 3=P f3, *Pseudomonas fluorescens* isolate 4= P f4, *Pseudomonas fluorescens* isolate 5=P f5, *Pseudomonas fluorescens* isolate 6= P f6, *Pseudomonas fluorescens* isolate 7=P f7, *Pseudomonas fluorescens* isolate 8= P f8, *Pseudomonas fluorescens* isolate 9=P f9, *Pseudomonas fluorescens* isolate 10=P f10, *Pseudomonas fluorescens* isolate 11= P f11, *Pseudomonas fluorescens* isolate 12=P f12, and maintained on Nutrient Agar slants for further assay for Siderophore Production.

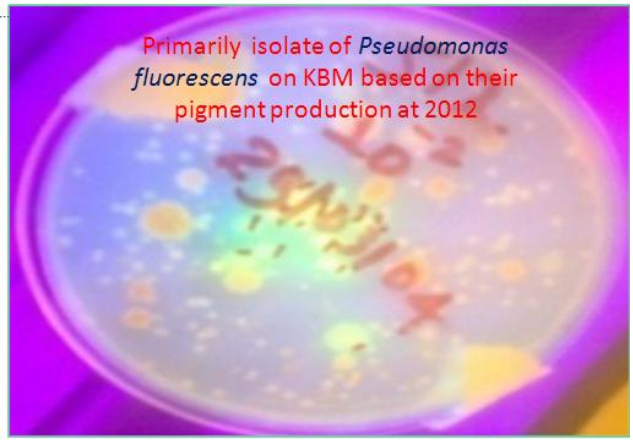


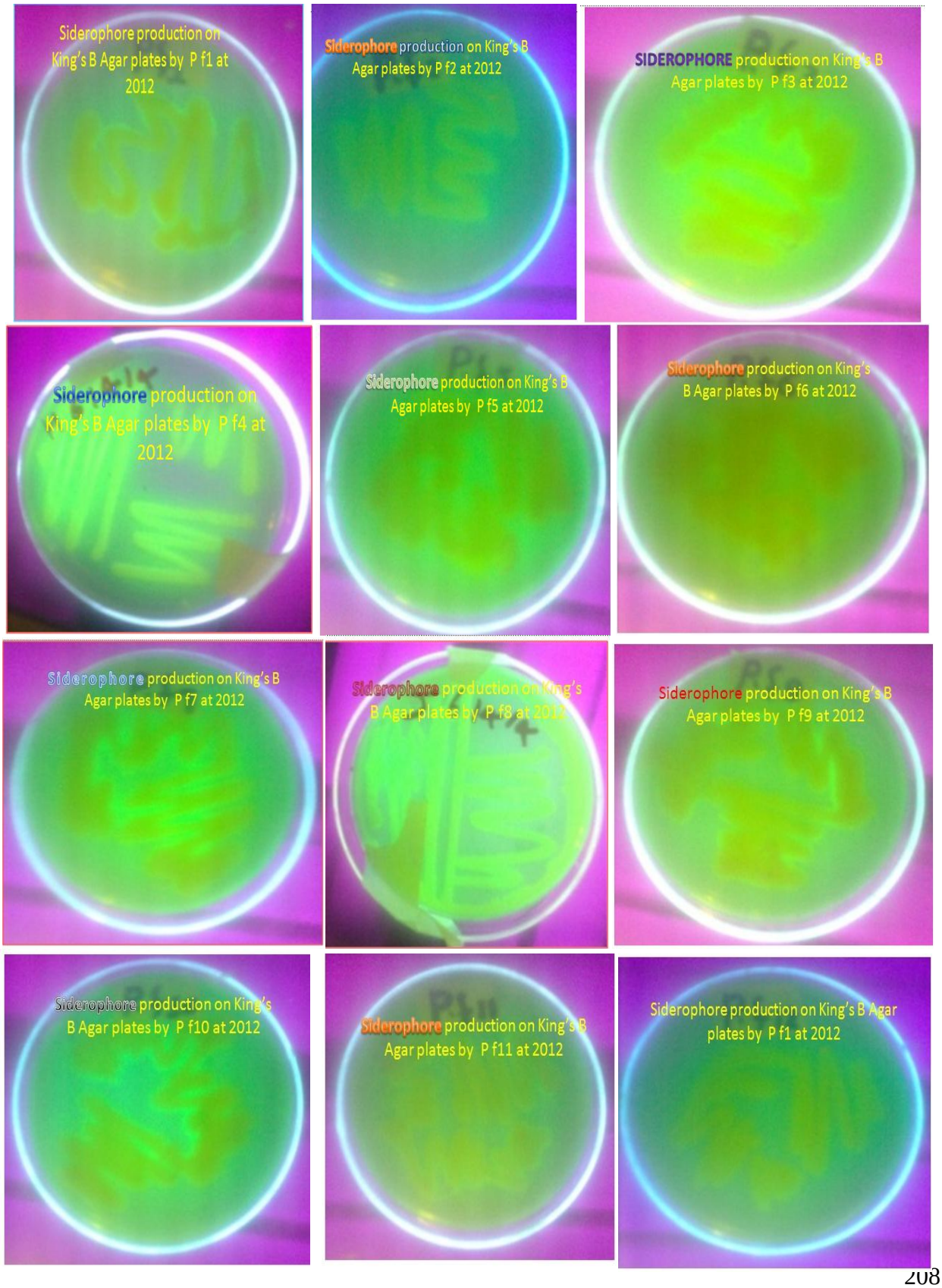
Figure 4. *Pseudomonas fluorescens* was screened based on their pigment production under UV light

Assay for siderophore Production

Production of Siderophore was exhibited by all the isolates of *Pseudomonas fluorescens* and colonies were exhibiting yellowish green pigment production on King's B Agar plates as shown in fig 6 and without using UV light, *Pseudomonas fluorescens* isolates had white colony on streaked King's B Agar as indicated in fig 5.

Figure 5. *Pseudomonas fluorescens* isolates was seen on King's B Agar plates without under VU light they had white colony on plates.

Figure 6. Siderophore production by *Pseudomonas fluorescens* isolates on King's B Agar plates when observed Under UV light at 365 nm.



In this study, the qualitative estimation of siderophores by *Pseudomonas fluorescens* isolates showed that they were powerful producer of siderophores under limited iron on King's B medium. The production of siderophores by *Pseudomonas fluorescens* isolates indicated that these bacteria isolates can be used as a biocontrol against soil borne phytopathogens. Similarly, Ramyasmruthi et al. (2012) reported that *Pseudomonas fluorescens* as siderophore producer on King's B medium. *P. fluorescens NCIM* 5096 was able to give higher yields of siderophores under iron stress conditions (Sayyed et al., 2005). Siderophores provide a competitive advantage to producer organism over fungal pathogens for the absorption of available iron (Jeffrey et al., 1999). The role of siderophores in the control of diseases has been well documented by Baker et al. (1986). In the aerobic environment, iron occurs principally as Fe^{3+} and is likely to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to microorganisms. To acquire sufficient iron, the most commonly found strategy in bacteria is the secretion of siderophores, low-molecular weight compounds with high affinity for Fe^{+3} with association constants for complexing iron (Nielands, 1981), which are produced under limiting concentrations of iron. These compounds are able to transport this element inside the iron starved cells for metabolic functions (Press et al., 2001).

To summarize up based on present studies, *Pseudomonas fluorescens* isolates under investigation possess a variety of promising properties which make them better siderophores producer that are capable of chelating iron and use as biocontrol. *Pseudomonas fluorescens* have a potential to produce siderophores molecule may use in Iron purification industry. This study may also need further study to identify the gene of these isolate that responsible for production of siderophores molecule.

ACKNOWLEDGMENTS

I am grateful thanks to Addis Ababa University, Department of Microbial, Cellular, and Molecular Biology, College of Natural Sciences which are giving facilities to conduct this study.

REFERENCES

Alexander, D.B. and Zuberer, D.A. (1991). Use of chromeazurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soil 2: 39-45.

Anderson, L.M., Stockwell, V.O. and Loper, J.E. (2004). An extracellular protease of *Pseudomonas fluorescens* inactivates antibiotics of *Pantoea agglomerans*. Phytopathol. 94: 1228-1234.

Atkin, C. L. and Neilands, J. B. (1968). Rhodotorulic acid, a diketopiperazine dihydroxamic acid with growth factor activity. Biochem. 7: 3734-3739.

Baker, R., Flad, Y. and Sneh, B. (1986). *Physical, Biological and Host Factors in Iron Competition in Soils in Swinburne TR (Ed) Iron Siderophores and Plant Diseases*. Plenum press, New York.

Baysse, C., Matthijs, S., Schobert, M., Layer, G., Jahn, D. and Cornelis, P. (2003). Co-ordination of iron acquisition, iron porphyrin chelation, and iron-protoporphyrin export via the cytochrome c biogenesis protein CcmC in *Pseudomonas fluorescens*. Microbiol. 12: 3543-3552.

Budzikkiewicz, H. (1992). Secondary metabolites from fluorescent Pseudomonas. Microbiol. Rev.104: 209-228.

Couillerot, O., Prigent-Combaret, C., Caballero-Mellado, J. and Moe¨nne-Loccoz, Y. (2009). *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. Lett. Appl. Microbiol. 48: 505–512.

Duhme, A.K., Hider, R.C., Naldrett, M.J. and Pau, R.N. (1998).The stability of the molybdenum azotochelin complex and its effect on siderophore production in *Azotobacter vivelandii*. J. Biol. Inorg. Chem. 3: 520-526.

Ferna´ndeza, D.U., Fuchsa, R., Schäfera, M., Budzikiewicza, H. and Meyer, J.M. (2003). The pyoverdin of *Pseudomonas fluorescens G173*, a novel structural type accompanied by unexpected natural derivatives of the corresponding. Z. Naturforsch. 58: 1-10.

Haas, D. and De´fago, G. (2005). Biological control of soil borne pathogens by fluorescent *Pseudomonads*. Nat. Rev. Microbiol. 3: 307–319.

Hofte, M., Boelens, J. and Verstraete, W. (1992). Survival and root colonization of mutants of plant growth promoting pseudomonads affected in siderophore biosynthesis or regulation of siderophore production. J. Plant Nutr. 15: 2253-2262.

Indiragandhi, P., Anandham, R., Madhaiyan, M. and Sa, T.M. (2008). Characterization of plant growth-promoting traits of bacteria isolated from larval guts of diamondback month *Plutella xylostella* Lepidoptera: Plutellidae. Curr. Microbiol. 56: 327-333.

Jeffrey, P., Gianinazzi, S., Perotto, S., Turnau, K. and Barea, J.M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol. Fertil. Soil 37: 1-16.

Keller-Schierlein, W. and Diekmann, H. (1970). Stoffwechselprodukte von Mikroorganismen. 85 Mitt. Zur Konstitution des Coprogens. Helvetica chimica acta 53: 2035-2044.

Kiely, P.D., Haynes, J.M., Higgins, C.H., Franks, A., Mark, G.L., Morrissey, J.P. and O'Gara, F. (2006). Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. Microbiol. Ecol. 51: 257–266.

King, E.O., Ward, M.K. and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanine and fluorescein. J. Lab. Clin. Med. 44: 301-307.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M. N. (1980b). Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 268: 885-886.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. (1980a). Pseudomonas siderophores: A mechanism explaining disease suppressive soils. Curr. Microbiol. 4: 317-320.

Leong, S. A. and Expert, D. (1990). Siderophores in plant pathogens interaction. Plant Microbiol. Interact. 3: 62-83.

Loper, J.E. and Henkels, M.D. (1997). Availability of iron to *Pseudomonas fluorescence* in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. Appl. Environ. Microbiol. 63: 99-105.

Maurer, B., Muller, A., Keller-Schierlein, W. and Zaner, H. (1968). Ferribactin, ein siderochrom aus *Pseudomonas fluorescens* Migula. Arch. Microbiol. 60: 326-339.

Meyer, J. M. (2000). Pyoverdins: Pigments siderohores and potential taxonomic markers of fluorescent Pseudomonas species. Microbiol. 174: 135-142.

Meyer, J. M. (1977). Pigment fluorescent et metabolisme du fer chez *Pseudomonas fluorescens*. d'Etat, Strasbourg.

Neilands, J. B. (1981). Microbial iron compounds. Annu. Rev. Biochem. 50: 715-731.

Neilands, J. B. (1974). *Microbial Iron Metabolism*. Academic Press, New York.

Pal, R. P. and Gokarn, K. (2010). Siderophores and pathogenecity of microorganisms. J. Biosci Tech. 1: 127-134.

Peix, A., Ramirez-Bahena, M. H. and Velazquez, E. (2009). Historical evolution and current status of the taxonomy of genus *Pseudomonas*. Infect Genet Evol. 6: 1132-1147.

Press, C.M., Looper, J.P. and Klopper, J.W. (2001). Role of iron in rhizobacteria mediated induced systemic resistance of cucumber. Phytopathol. 91: 593-598.

Ramyasmruthi, S., Pallavi, O., Pallavi, S., Tilak, K. and Srividya, S. (2012). Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from Solanaceae rhizosphere effective against broad spectrum fungal phytopathogens. Asian J. Plant Sci. Res. 2: 16-24.

Saharan, B.S. and Nehra, V. (2011). Plant growth promoting rhizobacteria: A critical review. Life Sci. Medicine Res. 21: 1-30.

Sayyed, R.Z., Badgujar, M. D., Sonawane, H.M., Mhaske, M.M. and Chincholkar, S. B. (2005). Production of microbial iron chelators (siderophores) by fluorescent *Pseudomonads*. Indian J. Biotechnol. 4: 484-490.

Subba Rao, N.S. (1999). *Soil Microbiology* (Fourth Edition of Soil Microorganisms and Plant Growth). Science Publishers, Inc., New York.

Villacieros, M., Power, B., Sanchez-Contreras, M., Loret, J., Oruzebal, R.I., Martin, M., Franandez-Pinas, F., Bouile, I., Whelan, C., Dowling, D.N. and Rivilla, R. (2003). Colonization behaviour of *Pseudomonas fluorescens* and *Sinorhizobium meloti* in the alfalfa (*Medicago sativa*) rhizosphere. Plant Soil 251: 47-54.

Zalkin, A., Forrester, J. D. and Templeton, D. H. (1966). Ferrichrome A tetrahydrate. Determination of crystal and molecular structure. J. Am. Chem. Soc. 88: 1810-1814.