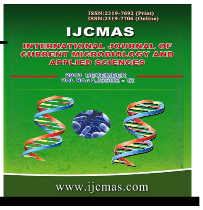
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 12 (2013) pp. 303-312**
http://www.ijcmas.com



Review Article

Bacterial Siderophore and their Application: A review

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ABSTRACT

Under iron restricted condition many bacteria produced iron chealating molecules called siderophore. Siderophore chealate iron and supply to bacterial cell by outer membrane receptors. A great variation is seen in siderophore structure produced by many bacteria. There are three main kinds of siderophores known as hydroxamate, catecholate and carboxylate. Siderophore production can be obtained under iron restrict media and many researcher have produced siderophore from bacteria on succinate media. Siderophore and their derivative have large application in agriculture as to increase soil fertility and biocontrol for fungal pathogen. In medicine the most important application is selective drug delivery, a Trojan horse strategy, to defeat drug resistant bacteria. Siderophore also used to reduce the level of metal contamination in environment specifically from soil and water.

Keywords

Bacteria;
Siderophore;
Application
Trojan horse
strategy.

Introduction

Iron is a vital element require by all living organisms for many cellular processes such as electron transport chain and as a cofactor for many enzymes (Litwin and Calderwood, 1993). Microorganisms growing under aerobic conditions need iron for a variety of functions including reduction of oxygen for the synthesis of ATP, for formation of heme and for other essential purposes.

The aerobic atmosphere of the planet has caused the surface iron to oxidized to insoluble oxyhydroxide polymer and reduced the level of free iron, therefore microorganism adopted a way for iron acquisition by producing iron chelating molecule i.e. siderophore. Siderophore are low molecular weight (< 10 KD) iron chelating compounds synthesized by many bacteria *Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum* and *Rhizobium* (Glick *et al.*, 1999, Loper *et al.*, 1999), in large quantity under iron limited conditions (Neilands, 1981). Siderophore forms complex with free iron and transport it into the cell by membrane receptor molecules, these molecules are encoded by five genes in operon which is turned off when sufficient iron has been taken into the cell (Lewin, 1984). Some bacteria produce one or more siderophores which can be utilized by other microorganism for iron and other metals acquisition. This property of siderophore increased their application, also siderophore have been related to virulence mechanisms in microorganism pathogenic to both animals and plants. In addition, they have applications in clinical, agriculture and environmental fields. At present nearly 500 siderophores are reported from selected microorganisms. A great variation is seen in siderophore structure from one species to another. There are three main kinds of siderophore known as hydroxamate, catacholate and carboxalate.

Types of siderophore

Hydroxamate siderophore

Hydroxamate siderophore are produced by bacteria and fungi. Most hydroxamate groups, C (=O)N-(OH) R, where R is an amino acid or a derivative. Each hydroxamate group provides two oxygen molecules, which form a bidentate ligand with iron. Therefore, each siderophore forms a hexadentate octahedral complex with Fe^{3+}. Hydroxamate siderophores usually show strong absorption between 425 and 500 nm when bound to iron. Ferrichrome produced by the fungus *Ustilago sphaerogena*, was the first siderophore to be isolated and shown to be a growth factor for other microorganisms (Messenger and Ratledge, 1985). Ferribactin produced by *Pseudomonas fluorescens* is known to be a hydroxamate. Gonobactin and nocobactin produced in small quantities by *Neisseria gonorrhoeae* and *N. meningitids* are also hydroxamates. The hydroxamate siderophores was detected by Neiland‘s spectrophotometric assay (Neilands, 1981).

Catecholate (Phenolates) siderophore

Enterochelin the cyclic trimester of 2, 3-dihydroxybenzoylserine, is produced by *E. coli*, *S. typhimurium* and *K. pneumonia* and is the prototype of the catecholate siderophore. Each catecholate group provides two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed as in the case of the hydroxamate siderophores. Linear catecholate siderophore are also produced in certain species. Agrobactin and parabactin are produced by *Agrobacterium tumefaciens* and *Paracoccus denitrificans* respectively. *Erwinia carotovora* produced catecholates while *Pseudomonas* produced a mixed catecholate-hydroxamate siderophore (Leong and Neilands, 1982). The catecholate nature of the siderophore is also detected by Neilands spectrophotometric assay (Neilands, 1981), Formation of wine coloured complex with $FeCl_3$ that absorbs maximally at 495 nm, indicates catecholate nature of siderophores.

Carboxylate (complexones) siderophore

The universal assay for siderophore detection (Schwyn and Neilands, 1987) has facilitated the detection of siderophore that are neither catecholates nor hydroxamates. The best characterized carboxylate type siderophore with a novel structure is rhizobactin. Rhizobactin is produced by *Rhizobium meliloti* strain DM4 and is an amino poly (carboxylic aci) with ethylenediaminedicarboxyl and hydroxycarboxyl moieties as iron-chelating groups. Staphyloferrin A, produced by *Staphylococcus hyicus* DSM20459, is another member of this class of complexon siderophores. Satphyloferrin A consists of one D-ornothine and two citric acid residues linked by two amide bonds.

Siderophore transport in bacteria

Siderophore-mediated iron uptake in microorganisms is both a receptor- and an energy-dependent process (Sigel and Sigel, 1998). Such systems have been well studied in *Escherichia coli* (Wandersman and Delepelaire, 2004). Siderophores are part of a multi-component system for transporting ferric iron into a cell. Other components include a specific outer membrane receptor protein Fec A, Fep A and TonB-ExbB-ExbD protein complex in the inner membrane, a periplasmic binding protein, and an inner membrane ATP-dependent Fec CDE- Fep CDE protein shown in (Fig. 1). Under iron deficiency bacteria synthesize siderophore and increase number of receptor molecules once the siderophore excreted outside of cell thorough membrane receptor it bind with iron complex and transport the iron in to the cell via Fec A and Fep A outer membrane receptor(OM), after it transported to Fec C,D,E and Fep C,D,E so called ABC-Transporter systems (from ATP-binding cassette) (Davidson and Nikaido, 1991, Boos *et al*., 2001) assembled of two proteins, one to span the membrane acting as a permease and a second one which can hydrolyse ATP to provide the energy for transport. Later siderophore iron complex release in cytoplasm with the help of membrane protein Ton B (Fig. I). In the cell cytoplasm, the iron released from the complex by a mechanism which is still in doubt: it may involve hydrolytic destruction of the siderophore molecule or the reduction of Fe^{3+} by a NAD (P) H-linked siderophore reductase or Ent A,B,C,D protein. The resulting Fe^{2+} does not have a high affinity for siderophore and therefore dissociated from the complex.

Siderophore production and extraction

Siderophore can be produced using iron restricted medium, However many researcher have produced bacterial siderophore by using succinate medium (Mayer and Abdullah, 1978) the fermented succinate broth showing siderophore production shown in (Fig. 2). After completion of incubation the siderophore were extracted by Page and Tingerstrom (1988) method and the crude siderophore crystals obtained by solvent extraction method shown in (Fig. 3).

Application of siderophore

Siderophore is biological molecule produced by various bacteria having wide application in various field such as agriculture to improve soil fertility and biocontrol, environmental application and medicinal application shown in (Fig. 4).

Agricultural application

In agriculture inoculation of soil with *Pseudomonas putida*, which produce pseudobactin, increases growth and yield of various plants (Kloepper *et al.*, 1980).Their plant growth promoting activities include production of HCN, siderophores, protease, antimicrobials, phosphate solubilizing enzymes (Chaiharn *et al*., 2008). Powell *et al*. (1980) have shown that hydroxamate siderophores are present in various soils and they are also produced in aquatic environments. Further excessive accumulation of heavy metals is toxic to most plants and contaminates the soil which result decreased soil microbial activity and soil fertility, and yield losses (McGrath *et al*., 1995). In this concern hydroxamate type siderophore present in soil play important role to immobilize the metals. Burton *et al*. (1954) had shown

Figure.1 Mechanism of Siderophore mediates iron transport in bacteria.

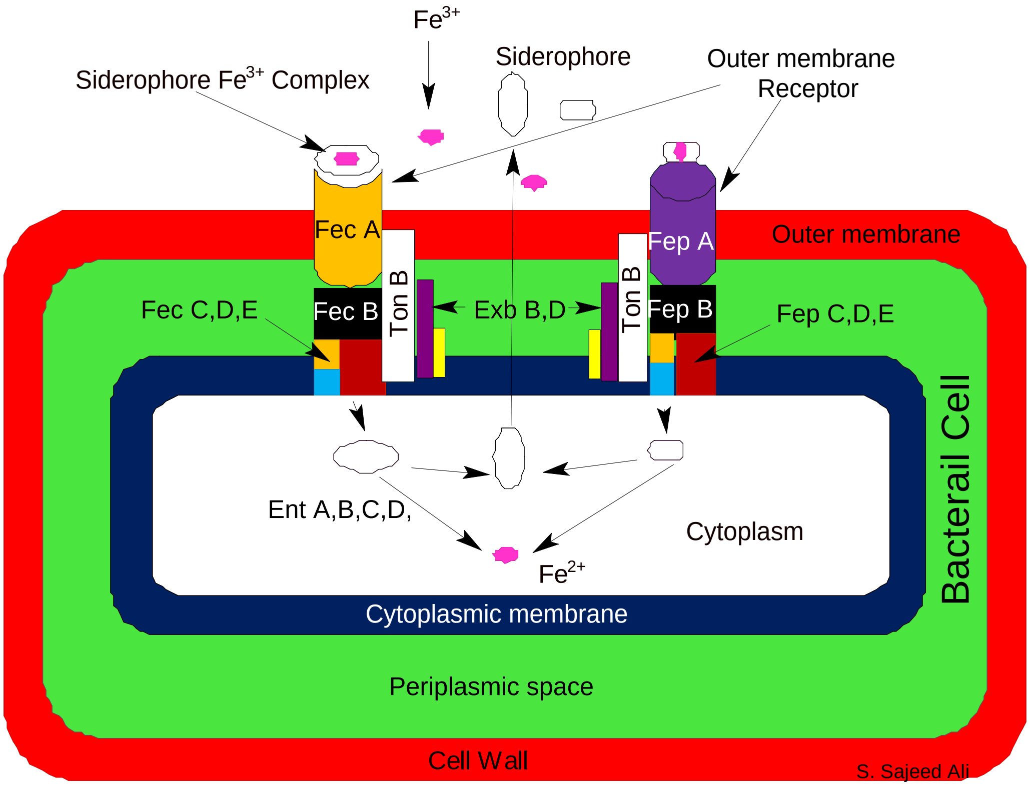
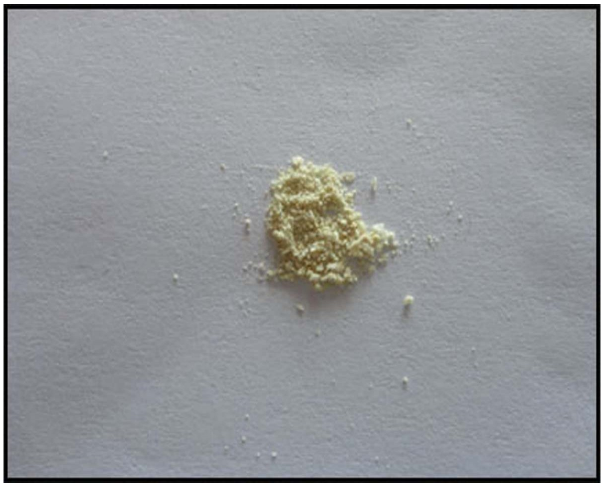


Figure.2 Crude Siderophore crystals



that some microbes synthesize siderophores whilst others use them without synthesizing any.

Biocontrol agent

Many bacteria suppress the growth of deleterious microorganism by production of siderophore, antibiotics, and cyanide (Edi Husane, 2005). Siderophores are themselves growth inhibitors of various phytopathogenic fungi, such as *Phytophthora parasitica* (Seuk *et al*., 1988), *Phythium ultimum* (Hamdan *et al.*, 1991), *Fusarium oxysporum veri dianthi* (Buysens *et al*., 1996) and *Sclerotinia sclerotiorum* (Mc Loughlin *et al*., 1992). Kloepper *et al*. (1980) were the first to demonstrate the importance of siderophore production as a mechanism of biological control of *Erwinia carotovora* by several plant-growthpromoting *Pseudomonas fluorescens* strains A1, BK1, TL3B1 and B10. And, a direct correlation was established *in vitro* between siderophore synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of *F. oxysporum* (Elad and Baker, 1985, Sneh *et al*., 1984). As with the antibiotics, mutants incapable of producing some siderophores, such as pyoverdine, were reduced in their capacity to suppress different plant pathogens (Keel *et al.*, 1989, Loper and Buyer, 1991).

Environmental applications

The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Ni. Metals are natural components in soil with a number of heavy metals being required by plants as micronutrients. However, pollution of biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution. Heavy metal contamination to water and soil poses a major environmental and human health problem. Siderophores and other naturally occurring ligands may therefore affect actinide mobility in waste repositories and in the environment and may also used to treat radioactive waste prior to storage or to decontaminate soils and water (Ruggiero *et al*., 2000; *Von Gunten* and *Benes*, 1995).

Medicinal application

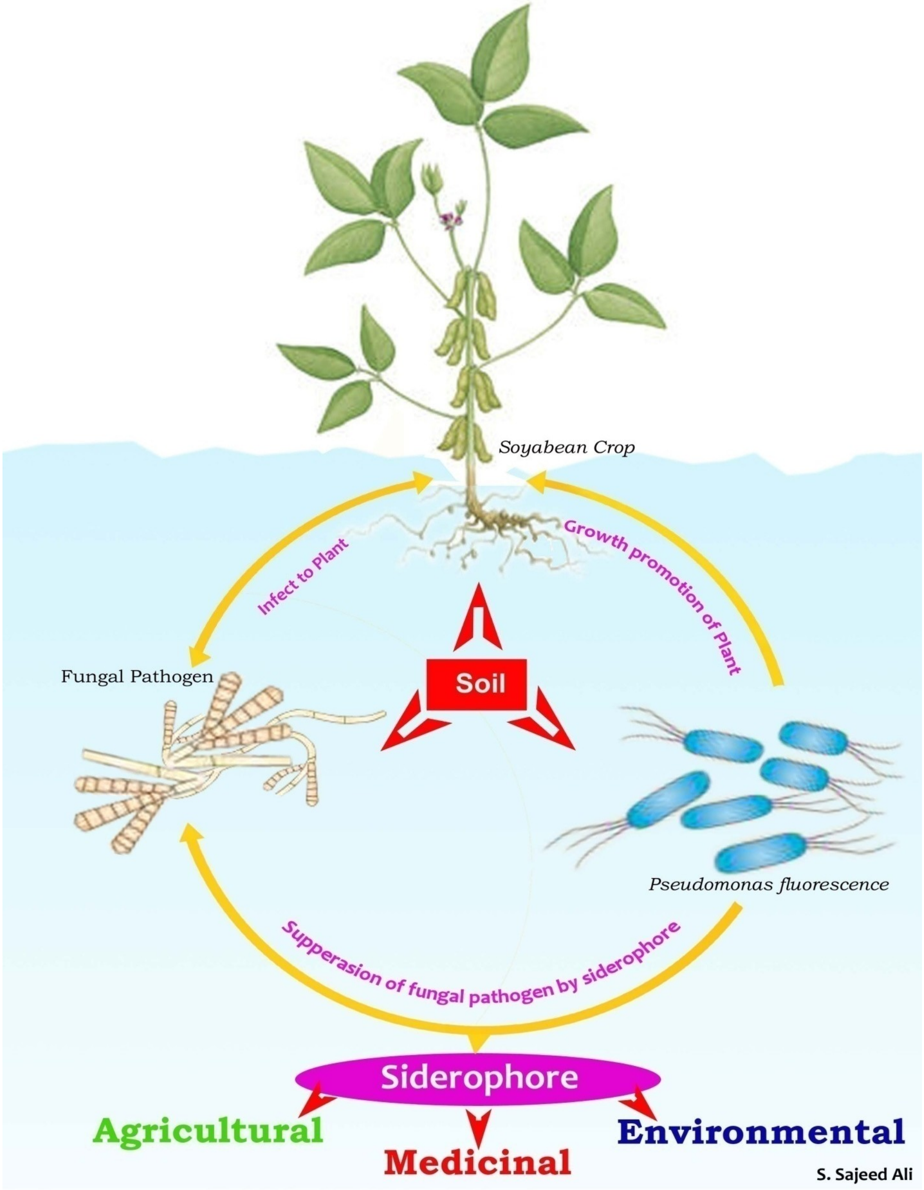
Iron overload diseases, ß-thalassemia

In the treatment of β-thalassemia and certain other anemias, periodic whole blood transfusions are required (Hershko *et al.*, 2002). Since there is no specific physiological mechanism for the excretion of iron in man, continued transfusion therapy leads to a steady buildup of iron. These iron excesses, as well as the primary iron overload diseases such as hemochromatosis and hemosiderosis, and accidental iron poisoning, require the removal of iron from the body, especially from the liver. Such disease can be efficiently treated with siderophore based drug and siderophore act as principal model (Pietrangelo, 2002). Desferrioxamine B has also found therapeutic application for various pathological conditions due to aluminum overload (Ackrill *et al.*, 1980). Accumulation of this toxic metal is frequently observed in chronically dialyzed patients who have lost the ability to clear via renal excretion. Desferrioxamine B has also been recommended for the diagnosis of such an overload state.

Infection

Iron is abundant in the human body, but it is bound to intracellular and extracellular

Figure.4 Application of siderophore



components (transferrin, lactoferrin, ferritin; hemo-proteins). This strict iron homeostasis leads unavailability of free iron for pathogenic bacteria in host body. Most aerobic, facultative anaerobic, and saprophytic microorganism have ability to produce high-affinity iron binding compounds, termed as siderophores, that are capable of chelating ferric iron and that allow its assimilation through cell surface receptors, therefore siderophore production contribute to bacterial virulence. It is thought that many pathogenic microorganisms acquire their essential iron from their hosts by this means (Litwin and Calderwood, 1993, Payne, 1993, Wooldridge and Williams, 1993).

Trojan horse antibiotics

Siderophores can be used for selective delivery of antibiotics in antibiotic resistant bacteria. It is the potentially powerful application that uses the iron transport abilities of siderophores to carry drugs into cells by preparation of conjugates between siderophores and antimicrobial agents ("Trojan Horse" strategy). Nature has provided examples for siderophore-antibiotics such as albomycins (Benz *et al.,* 1982). Ferrimycins (Bickel *et al.,* 1966) or salimycins (Vertesy *et al.,* 1995). The albomycins use a part of the ferrichrome structure for Fe3+ chelation, attached via a serine spacer to a toxic molecule. Several microorganisms introduce albomycin through the ferrichrome uptake system into the cell, where the toxic part is released enzymatically with detrimental effects to the cell. Similarly, ferrimycin has attached a moiety with antibiotic activity to ferrioxamine B by an amide link. Salimycins use a dicarboxylic acid as a spacer between the trihydroxamate siderophore and an aminoglycoside antibiotic. The occurrence of natural siderophore-antibiotic has opened the way to produce synthetic Trojan Horses.

Transuranic elements

The development of electricity generation by nuclear energy has led to increased human exposure to transuranic elements such as aluminium. Siderophore can be used to remove such elements from the body. Investigation has been carried out to evaluate the capacity of siderophore in removing such elements from the body. Administration of desferioxamine a form of siderophore lowers the level of aluminum in the body and relives the symptoms of the disease (Arze *et al.,* 1981).

Siderophores and MRI

For improved contrast enhancing for magnetic resonance imaging, different paramagnetic ions like Mn2+, Fe3+, and Gd3+ have been used. The Gd3+ is particularly well suited as contrast agent in diagnostoc medical MRI due to its high magnetic moment and favorable electronic relaxation rate, However, Gd3+ is highly toxic at concentrations required for MRI. Therefore, chelators are required that prevent release of the free cation in vivo. Again, siderophores and synthetic analogs thereof serve as principal models for such chelators (Doble *et al*., 2003).

Iron chelators and cancer

Siderophore potential used as iron chelators in the treatment of cancers e.g. Dexrazoxane, O-trensox, desferriexochelins, desferrithiocin, tachpyridine, have been found in cancer therapy (Miethke and Marahiel, 2007). Also siderophore used for the clearance of non-transferrin bound iron in serum which occurs in cancer therapy as a result of some chemotherapies (Chua *et al*., 2003).

Anti-Malarial

Some siderophore have been found to be useful in the treatment of malaria caused by *Plasmodium falciparum*. Siderophore produced by *Klebsiella pneumoniae* act as antimalarial agent (Gysin *et al.,* 1991). Desferrioxamine B produced by *Streptomycespilosus* (Now produced by chemical synthesis also) is active against *P. falciparum in vitro* as well as *in vivo*. Siderophore enters inside *P. falciparum* cell and causes intracellular iron depletion. The same siderophore was shown to inhibit growth of *Trypanosoma brucei*, another protozoic parasite causing sleeping sickness in human bloodstream (Breidbach *et al*., 2002)

Conclusion

Under aerated conditions at neutral to alkaline pH, inorganic iron is extremely insoluble and its concentration is less than optimal for bacterial growth. To acquire iron bacterial cell produce siderophore. There is an enormous scope for the application of microbial siderophores for the sustainability of humans, animals and plants. Currently the applications of siderophores in medicine, agricultural and environmental sector are reported in some extent. But the application of siderophore research is not at all initiated in various field of microbiology. So, there is a need to discover siderophores from normal and also extremophiles in the ecosystems like deep sea, desert and forest to exploit their applications for welfare of all living beings as well as for environment.

References

Ackrill, P., Raiston, A.J., Day, J.P and Hodge K.C. 1980. Successful removal of aluminum from patients with encephalopathy. Lancet. 2, 692-693.

Arze, R. S., Parkinson, I. S., Cartilidge, N. E. F., Britton, P. and Ward, M. K. 1981. Reversal of aluminium dialysis encephalopathy after desferrioxamine treatment. Lancet. 2, 1116.

Benz, G., Schroder, T., Kurz, J., Wunsche, C., Karl, W.,Steffens, G., Pfitzner, J., Schmidt, D. 1982. Konstitution der Desferriform der Albomycine d1, d2 and e. Angew. Chem. 94, 552-553 and Suppl. 1322-1335.

Bickel, H., Mertens, P., Prelog, V., Seibl, J., Walser, A. 1966. Über die Konstitution von Ferrimycin A1. Tetrahedron Suppl. 8/I, 171-179.

Boos, W., Eppler,T. 2001.*Prokaryotic Binding Protein-Dependent ABC Transporters*, in '*Microbial Transport Systems'*, ed. G. Winkelmann, Wiley-VCH, Weinheim, p77-114.

Breidbach, T., Scory, S., Krauth-Siegel, R.L., Steverding, D. 2002. Growth inhibition of bloodstream forms of *Trypanosoma brucei* by the iron chelator deferoxamine. Int J Parasitol. 32(4), 473 - 9.

Burton, M.O., Sowden and Lochhead, A.G. 1954. The isolation and nature of the terregens factor. Canadian Journal of Biochemistry and Physiology. 32, 400-406.

Buysens, S., Heungens, K., Poppe, J., Hofte, M. 1996. Involvement of Pyochelin and pioverdin in suppression of *Pseudomonas aeruginosa* 7NSK2. Applied and Environmental Microbiology 62(3), 865-871.

Chaiharn, M., Chunhaleuchanon, S., Kozo, A., Lumyong, S. 2008. Screening of rhizobacteria for their plant growth promoting activities. J. KMITL Sci. Tech 8, 18-23.

Chua, A.C., Ingram, H.A., Raymond, K.N. and Baker, E. 2003. Multidentate pyridinones inhibit the metabolism of nontransferrin-bound iron by hepatocytes and hepatoma cells. European Journal of Biochemistry. 270, 1689- 1698.

Davidson, A. L. and Nikaido, H. 1991. Purification and characterization of the membrane-associated components of the maltose transport system from Escherichia coli. J. Biol.Chem. 266, 8946-8951.

Doble, D. M. J., Melchior, M., OSullivan, B., Siering, C., Xu, J., Pierre, V. C., Raymond, K. N. 2003. "Toward Optimized High-Relaxivity MRI Agents: The Effect of Ligand Basicity on the Thermodynamic Stability of Hexadentate Hydroxypyridonate/Catecholate Gadolinium(III) Complexes" *Inorg. Chem. 42*, 4930-4937.

Edi Husen. 2003. Screening of soil bacteria for plant growth promotion activities in vitro. Indo.J. Agric. Sci 4(1), 27-31.

Elad, Y., and Baker, R. 1985. Influence of trace amounts of cations and iderophore-producing pseudomonads on chlamydospore germination of *Fusarium* *oxysporum*. Ecol. Epidemiol. 75, 1047-1052.

Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M. 1999. Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria. Imperial College Press. London.

Gysin. Jurg., Crenn., Yves., Pereira da silva., Luiz., Breton., Catherine. 1991. Siderophores as anti parasitic agents. US patent. 5, 192-807.

Hamdan, H., Weller, D., Thomashow, L. 1991. Relative importance of fluorescens siderophores and other factors in biological control of *Gaeumannomyces graminis* var. Tritici by *Pseudomonas fluorescens* 2-79 and M4-80R. Applied and Environmental Microbiology 57(11), 3270-3277.

Hershko, C., Link, G., and Konijn, A. M. 2002. Cardioprotective Effect of Iron Chelators, in Iron Chelation Theraphy , ed. Hershko, C., ed, Kluwer Academic / Plenum Publishers, New York, Vol. 509, 1 Ed pp. 77-89.

Keel, C., Voisard, C., Berling, C. H., Kahir, G., and Defago, G. 1989. Iron sufficiency is a prerequisit for suppression of tobacco black root rot by *Pseudomonas fluorescnes* strain CHA0 under gnotobiotic contiditions. Phytopathology. 79, 584-589.

Kloepper, J.W., Leong, J., Teinize, M and Schroth, M.N. 1980. Enhanced plant growth by siderophores produced by plantgrowth promoting rhizobacteria. Nature. 286, 885-886.

Leong, S.A. and Neilands, J.B. 1982. Siderophore production by phytopathogenic microbial species. Arch. Biochem. Biophys. 281, 351-359.

Lewin, 1984. How microorganism transport Iron. Science. 225, 401-402.

Litwin, C. M., and Calderwood, S. B. 1993. Role of iron in the regulation of virulence genes. Clin. Microbiol. Rev. 6, 137-149.

Litwin, C.M., and Calderwood, S.B. 1993. Role of iron in regulation of virulence genes. Clin. Microbiol. Rev. 6, 137-149.

Loper, J. E., and Buyer, J. S. 1991. Siderophores in microbial interactions of plant surfaces. Mol.Plant-Microbe Interact. 4, 5-13.

Loper, J.E., Henkels, M.D. 1999. Utilization of heterologous siderophore enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Applied Environmental Microbiology. 65, 5357-5363.

Meyer, J.M., Abdallah, M.A. 1978. The florescent pigment of *Pseudomonas fluorescens*. Biosynthesis, purification and physical-chemical properties. J. Gen. Microbiol. 107, 391-328.

Mc Loughlin, T., Quinn, J., Bettermann, A., Bookland, R. 1992. *Pseudomonas cepacia* suppression of sunflower. *Pseudomonas cepacia*. Wilt fungus and role of anti-fungal compounds in controlling the disease. Applied and Environmental Microbiology. 58(3), 1760-1763.

McGrath, S.P., Chaudri, A.M., Giller, K.E., 1995. Long-term effects of metals in sewage sluge on soils, microorganisms and plants. J. Ind. Microbiol. 14(2), 94-104.

Messenger, A.J.M., Ratledge, C. 1985. Siderophores. Comprehensive Biotechnology.3, Edited by M Moo-Young (Pergamon press, New York), pp. 275-295.

Miethke, M. and Marahiel, M. A. 2007. Siderophore-Based Iron Acquisition and Pathogen Control. Microbiol Mol Biol Rev. 71(3), 413–51.

Neilands, J.B. 1981. Microbial iron compounds. Annual Review of Biochemistry 50, 715- 731.

Page, W.J., and Tingerstrom, V.M. 1988. Dual regulation of catecholate siderophore biosynthesis in Azotobacter vinelandii by Fe and Oxidative stress. J.Gen. Microbiol. 134, 453-460.

Payne, S. M. 1993. Iron acquisition in microbial pathogenesis. Trends Microbiol. 1, 66-69.

Pietrangelo, A. 2002. Mechanism of iron toxicity, in Iron Chelation Theraphy ed. Hershko, C., ed,Kluwer Academic / Plenum Publishers, New York, Vol. 509, 1 Ed pp. 19-43.

Powell, P.E., Cline, G.R., Reid, C.P.P and

Szaniszlo, P.J. 1980.Occurrence of hydroxamate siderophore iron chelators in soils. Nature. 287, 833-834.

Ruggiero, C. E., M. P. Neu, J. H. Matonic, and S. D. Reilly. 2000. Interactions of Pu with desferrioxamine siderophores can affect bioavailability and mobility. Actinide Res. Q. 2000**,**16-18.

Schwyn, B. and Neilands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47-58.

Seuk, C., Paulita, T., Baker, R. 1988. Attributes associate with increased bio-control activity of fluorescent *Pseudomonads*. J. Plant Pathol. 4(3), 218-225.

Sigel, A. and Sigel,G. 1998. Iron transport and storage in microorganisms, plants, and animals, metal ions in biological systems Vol. 35, Marcel Dekker, Basel.

Sneh, B., Dupler, M., Elad, Y., and Baker, R. 1984. Chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from *Fusarium* suppressive soils. Phytopathology. 74, 1115-1124.

Vértesy, L., Aretz, W., Fehlhaber, H.W., Kogler, H. 1995. Salimycin A-D, Antibiotoka aus *Streptomyces violaveus*, DSM 8286, mit Siderophor-Aminoglycosid-Struktur. Helv.Chim. Acta. 78, 46-60.

Von Gunten, *H. R.* and *Benes*, *P*. 1995 Speciation of radionuclides in the environment. *Radiochim*. *Acta.* 69, 1-29.

Wandersman, C., and P. Delepelaire. 2004. Bacterial iron sources: from siderophores to hemophores. Annu. Rev. Microbiol. 58, 611-647.

Wooldridge, K.G., and Williams, P.H. 1993. Iron uptake mechanism of pathogenic bacteria. FEMS Microbiol. Rev. 12, 325-348.