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Microbial siderophore research: Reviewing their roles and applications

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Abstract

Under iron restricted conditions many bacteria produce iron chelating molecules called siderophores. Siderophores chelate iron and supply it to bacterial cells by outer membrane receptors. A great variation is seen in siderophore structure. The three main kinds of siderophores known are hydroxamate, catecholate and carboxylate. Siderophore production can be obtained under iron restricted media and many researchers have produced siderophore from bacteria on succinate media. Siderophores and their derivatives have large applications in agriculture as to increase soil fertility and also possess several biocontrolling abilities. In medicine the most important application is selective drug delivery, a Trojan horse strategy, to defeat drug resistant bacteria. Siderophores are also used to reduce the level of metal contamination in environment specifically from soil and water.

Keywords: chelate, Siderophores, biocontrolling, resistant.

Introduction

Iron is a vital element required by all living organisms for many cellular processes such as electron transport chain, DNA synthesis and as a cofactor for many enzymes (Litwin and Calderwood, 1993). Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil or sea is limited by the very low solubility of the Fe^{3+} ion. 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 insoluble oxyhydroxide polymer and reduced the level of free iron in the environment is present as various forms of insoluble iron oxides which are not easily accessible by bacteria. Therefore microorganism adopted a way for iron acquisition by producing iron chelating molecule i.e. siderophore. Siderophores are low molecular weight (< 10 KD) iron chelators of ferric iron ($Fe(III)$) that are synthesized under conditions of iron starvation by many bacteria *Pseudomonas*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Serratia*, *Azospirillum*, *Rhizobium* and *Actinomycetes* (Glick et al. 1999, Loper Henkels, 1999).

Most siderophores are excreted extracellularly to bind $Fe(III)$ and the ferric complexes, are recovered by bacteria via specialized 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. $Fe(III)$ is dissociated from the siderophore for use by the bacterium (Lewin, 1984). Siderophores are a structurally diverse group of molecules and can be classified on the basis of the chemical structure of the functional group that interacts with $Fe(III)$. The two most common classifications of siderophores are catechol and hydroxamate and some siderophores have a mix of groups, i.e. mixed carboxylate-hydroxamate, salicylate. Siderophores can also incorporate other, less common, functional groups that will complex with iron. Some bacteria produce one or more siderophores which can be utilized by other microorganism for iron and other metals acquisition. This property of siderophores increases their applications in clinical, agriculture and environmental fields. Siderophores have also been related to virulence mechanisms in microorganism pathogenic to both animals and plants.

Structural features of siderophores

About 500 low molecular weight structures with an extremely high affinity to $Fe(III)$ ions are identified. These molecules are synthesized by fungi, bacteria

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and some plants to increase their access to iron. They are referred to as siderophores. Their common feature is to form six co-ordinate octahedral complexes with ferric ion of great thermodynamic stability. As there are a number of different siderophore structures, it is quite difficult to categorize them. The structures stand for a typical property for a group of siderophore synthesizing organisms. (Albrecht-Gary & Crumbliss, 1988). While studying the siderophore structures the β -value and the pFe-values are also of importance.

The β -value measures the stability of the iron siderophore complex and is defined as: $\beta = [Fe_xLy]/[Fe]^x[L]^y$, where L represents the fully deprotonated siderophore. X and Y usually are 1, except for the tetradentate ligands, where X is 2 and Y is 3. β -values can only be compared within siderophores with the same number of binding sites.

The pFe-value is a measure of the overall efficiency of a siderophore and is defined as:

$-\log [\text{uncomplexed } Feaq^{3+}]$ at pH 7.4, 1 M total Fe^{3+} and 10 M total ligand concentration.

The binding sites very strongly determine the stability of the iron siderophore complexes. For all there are a lot of different siderophore structures, with three different moieties the binding sites of most siderophores can be described. These three moieties are: Hydroxamate, Catecholate and the Hydroxy-carboxylic acid group. The three binding sites always coordinate the iron with the lone pair of an oxygen atom.

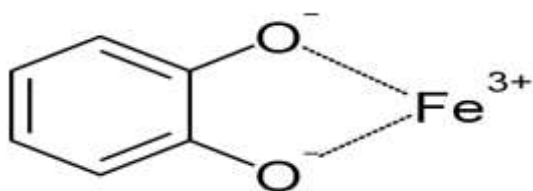


Fig. 1: Catecholate siderophore

As oxygen is a very hard donor ligand it shows a high affinity to the hard Fe(III) cation. This interaction enhances the strength of the coordination as well as the selectivity of the siderophore ligands for iron. As the oxygen atoms only coordinate when they are not protonated, pKa-values of the binding groups have a large influence on the effectivity of siderophores. The pKa values are not easily accessible and affected by the chain they are connected to. For hydroxamic acid a pKa-value in the order of 8 to 10 seems to be realistic in most cases. The acetohydroxamic acid which is a model compound for a mono hydroxamate ligand, has the pKa-value 9.29 (Kraemer, 2004).

For the catecholate a model monomer has been synthesized. For modelling the extreme cases, once a nitro-group in para-constellation is added and once a hydrogen atom 11 was connected. For the nitro group the resulting pKa-values for both alcohol groups are 6.69 and 10.83, respectively. The pKa-values imply the least partly deprotonated state of the hydroxamate and the catecholate groups in ambient pH (in the region of 7). Many studies indicate that all three groups are deprotonated at ambient pH especially in the presence of iron (Reid et al.,1993;Dhaungana et al.,2004)

Most of the presently known siderophores are hexadentate. This means that their binding sites are able to occupy six coordination sites of a metal. This phenomenon is intuitively understandable due to the fact that iron cations also have six coordination sites. This allows hexadentate siderophores to fully complex an iron cation. Two effects could help to give a scientifically based explanation. The effects are the “chelate effect” and the “concentration effect”. The chelate effect mainly says that an increased ligand denticity leads to an increase of complex stability. As some research says that it might be because of the relatively long connecting chains between the individual binding sites, which lead to an independent behaviour of each group b (Albrecht-Gary & Crumbliss, 1988; Boukhalfa et al., 2006).

The following table: 1 shows the three different types of arrangements that can be observed based on the various number of possibilities to connect the binding sites.

Table:1

Type of arrangement	Examples	Description
Linear Acyclic	Desferrioxamine B Rhodotorulic acid	The binding sites are placed on one acyclic chain. As a consequence, the chelation cavity for the centre iron atom is not fixed by molecule bonding and therefore not very stable.
Endocyclic	Ferrioxamine E, Alcaligin	In these siderophores the binding sites are positioned on a cyclic chain and point to the centre of the cycle. This constitution limits the flexibility of the siderophores configuration.
Exocyclic	Enterobactin, Ferrichrome	Here the binding sites are indirectly located on a cyclic basic-chain

(Albrecht-Gary & Crumbliss,1988; Renshaw et al., 2002, Kraemer et al 2004).

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 Fe³⁺. 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 & Rutledge, 1985)

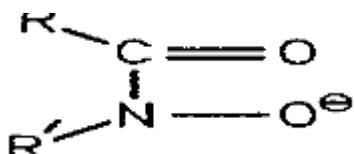


Fig. 2: Hydroxamate siderophore

Different types of hydroxamate siderophore produced by a few organisms is showed in table 2.

Table:2

Hydroxamate Siderophore	Organism
Ferribactin (Ferrichrome)	<i>Ustilago sphaerogena</i>
Gonobactin	<i>Neisseria gonorrhoeae</i>
Nocobactin	<i>Neisseria meningitidis</i>
Desferrioxamin B (Desferrioxamin)	<i>Streptomyces pilosus</i> <i>Streptomyces coelicolor</i>
Desferrioxamine E	<i>Streptomyces coelicolor</i>
fusarinine C	<i>Fusarium roseum</i>
Ornibactin	<i>Burkholderiacepacia</i>
Rhodotorulic acid	<i>Rhodotorula pilimanae</i>

Catechol siderophore

Each catechol 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.

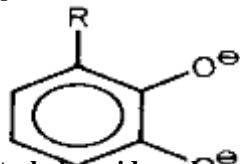


Fig. 3: Catechol siderophore

Siderophores show the maximum absorption at 495 nm which indicates catechol type of nature of

siderophores. Catechol siderophores may be of Cyclic or Linear type. Some are of the mixed group of catecholate-hydroxamate group (Leong & Neilands, 1982)

Table:3

Catechol siderophore	Organism
Enterochelin (cyclic trimer of 2, 3-Dihydroxybenzoylserine)	<i>Escherichia coli</i> , <i>Salmonella typhimurium</i> and <i>Klebsiella pneumonia</i>
Agrobactin (Linear catecholate)	<i>Agrobacterium tumefaciens</i>
Parabactin (Linear catecholate)	<i>Paracoccus denitrificans</i>

Carboxylate-Hydroxamate (Mixed group) siderophore

The universal assay for siderophore detection has facilitated the detection of siderophores that are neither catecholates nor hydroxamates, but shows the mixed group of hydroxycarboxyl moiety as iron chelating group, called as carboxylate-hydroxamate siderophore. Rhizobactin is produced by *Rhizobium meliloti* strain DM4 and is an amino poly (carboxylic acid) with ethylenediaminedicarboxyl and hydroxycarboxyl moieties as iron chelating groups. Staphyloferrin A, produced by *Staphylococcus hyicus* DSM20459, is another member of this class of complex siderophores. Staphyloferrin A consists of one Dornothine and two citric acid residues linked by two amide bonds. The bacterial siderophore pyoverdinin of *Pseudomonas putida* expresses one binding site of each of the three types. At pH-values from 9 to 11 the pyoverdinin coordinates in a hexadentate mode and forms very stable complexes. But at lower pH the hydroxy-carboxylic acid group soon starts to dissolve and thereby weakens the complex. This shows that the efficiency of a siderophore depends also on the environment into which it is released. Besides these very common binding sites there is also a variety of other less abundant types. Mugineic acid a common phytosiderophore, forms hexadentate complexes with a hydroxy-carboxylic acid group and two amino carboxylate groups. Rhizoferrin is a fungal siderophore that consists of two molecules of citric acid which are linked by an additional chain. The siderophore has two hydroxy-carboxylic acid groups and two carboxylic acid groups that can coordinate the iron. From the so far characterized fungal siderophores it is the only one that does not coordinate the iron with hydroxamate moieties

(Albert Gary and Crumbliss, 1998, Renshaw et al 2002).

Table: 4

Mixed siderophore	Organism
Azotobactin	<i>Azotobacter vinelandii</i>
Pyoverdine	<i>Pseudomonas aeruginosa</i>
Yersiniabactin	<i>Yersinia pestis</i>

Siderophore producing microorganisms

Rhizosphere species like *Pseudomonas*, *Bacillus*, *Clostridium*, *Azotobacter*, *Azospirillum*; Actinomycetes like *Streptomyces*, *Nocardia*; and Fungi like *Fusarium*, *Verticillium*, *Aspergillus* and *Penicillium* are the standard microorganisms which produce the different types of siderophores. Some pathogenic microorganisms species like *Salmonella*, *Serratia*, *Enterobacter* also shows the siderophore production. Other known bacteria producing siderophores belong to the strains of *Pseudomonas aeruginosa*, *Delftia acidovorans*, *Bacillus subtilis* and *Rhodococcus erythropolis*.

Production and extraction of siderophores

Biosynthesis and production of excess siderophores is not expected until the culture is stressed for Fe^{3+} ions. Even a small amount of ferric sulfate addition will inhibit siderophore production but promote culture density. Mechanism of iron regulated siderophoregenesis in enteric bacteria and *pseudomonas* sp. is known where transcription of iron regulated gene is under the negative control of fur (ferric uptake regulation) protein (repressor) with Fe^{2+} as an essential co-repressor. Best siderophore production is possible in minimal media excluding iron salts. In case of complex, a pretreatment of deferration is required. Defferation, removal of iron, can be achieved by shaking the media with a solution of 1% 8- hydroxyl quinolone in chloroform until the organic layer comes away clear. Finally medium should be extracted with pure chloroform to remove traces of chelating agent for heavy metals hence subsequent addition of other essential metals should be made for optimal growth under iron stress. To ease defferation while preparing complex media in large volumes, if one particular media component is known to carry iron contamination, separate defferation of this component can be considered (Rane et al 2005).

Detection of siderophores

Detection of pyoverdine siderophores of *Pseudomonas aeruginosa* is simple due to its particular yellow green fluorescence. Reaction of functional groups to produce a change in colour is a method for detection of colourless siderophores. The most primary method would be to add iron to the culture supernatant, resulting in colour formation. Although many siderophore are detected by this trick, it may ignore presence of some siderophores produced in small quantity. Several of the chemical methods listed below have been introduced which are highly sensitive chemical assays:

- 1) Ferric Chloride Reaction
- 2) Csaky Test
- 3) Arnow's Reaction
- 4) Periodate Oxidation of Hydroxamate
- 5) CAS (Chrome Azurol Sulfonate) Assay

Among these methods CAS assay is the well known universal chemical assay for the detection of siderophore. The chrome azurolsulfonate (CAS) assay was used for the detection of siderophores. This assay uses an iron-dye complex which changes color on loss of iron. Siderophores, which have more affinity for the iron than the dye, remove the iron, resulting in a change in color of the dye to orange. The iron-dye complex used to detect the siderophore thus can be incorporated into King's B medium.

Extraction and purification of siderophores

Siderophore are synthesized inside cells under iron-starved conditions and then secreted in the medium. (Rane et al 2005) Several chemical, physical and structural peculiarities of siderophores can be studied after its efficient extraction and purification. Several processes employed are:

Ethyl acetate Extraction

This method is specifically followed for catechol siderophores. Here the supernatant containing siderophores is extracted with ethylacetate. The separated organic solvent is then concentrated and washed with 0.1M Sodium citrate (pH5.5) and dried over anhydrous $MgSO_4$ and the precipitated with hexane (Chaudhari and Chincholkar 1998).

Chloroform-ethanol-Ether-Water Extraction

Repeated extraction of aqueous supernatant containing siderophores with chloroform-phenol (1:1) followed by dilution of organic extract with diethyl ether and organic extraction with 3-4 small volumes of water will bring most siderophores in aqueous solution (Jalal et al., 1984).

XAD-2 Extraction

In this method the culture supernatant, chelated by 2% FeCl₃ solution is passed through pre-washed (water, methanol, water) XAD-2 column. Pale coloured elute should be discarded. Any unbound impurities are washed by 2-5 column volumes of water. Siderophores with lipophilic properties and solubility in ethanol or methanol are best extracted by this method. (Emery 1965)

Gel filtration

For the purification of siderophores by this method culture supernatant is lyophilised and the residue dissolved in 5ml of deionized water and centrifuged to remove undissolved material. Then 2ml sample is applied to Sephadex G-25gel filtration Column equilibrated with water-ethanol (10:1,vol/vol) as a solvent. Fractions of 3 ml were collected and assayed for siderophores by CAS assay (Yang et al 1991).

Thin Layer Chromatography

TLC of samples containing phenolate type siderophores can be carried out using ascending chromatography at room temperature for 2 hrs. using ammonium formate and formic acid as solvent. (Gibson and Magrath 1969). For hydroxamate type siderophores solvent system N-butanol -acetic acid-water was used. The dries plates were then sprayed with 1% FeCl₃ for the detection of siderophores. (Menon 2005).

Ion Exchange Chromatography

Siderophores carrying charge can be purified by ion exchange chromatography. Depending on the charge present on these molecules CM-sephadex, CM-cellulose and DEAE-cellulose may be used for positively charged siderophores. While DEAE-sephadex are used for anionic siderophores. Siderophore mixture is applied onto swollen, washed and degassed support material in a column of suitable size. After application of mixture, column should be washed using water/buffer to elute unbound impurities and neutral or oppositely charged siderophore. Deionised water or chosen buffer can be used as a mobile phase. Bound siderophores may be eluted with a salt gradient (0 to 1M NH₄Cl/NaCl).

Paper Electrophoresis

It is employed for analysis of charge on siderophore Routine electrophoresis is carried out on Whatman 3M paper with 100 V field strength for 1 h using pH 5.0 (pyridine-acetic acid-water, and pH 2.0 (formic acid). Mobility of molecule is determined by charge/mass ratio.

Column Chromatography

Extracts obtained through Gel-filtration column or ion exchange column may be separated in preparative

silica gel column. The solvent of choice is usually chloroform-methanol-water. Individual bands coming out may contain overlapped fractions that should be rechromatographed to obtain complete separation (Jalal and Van der Helm 1991).

Siderophore-iron transport

In constructing a binding agent with requisite affinity/specificity for Fe(III), nature appears to have exceeded the free diffusion limit of the outer membrane of Gram-negative bacteria. Thus, enterobactin, the siderophore indigenous to *E. coli*, has a molecular weight of 669 while that of ferrichrome, produced by fungi and utilized avidly by *E. coli* and other bacteria, is 740. (Wandersman & Delepelaire, 2004). This exclusion-by-size has required the insertion in the outer membrane of specialized receptors. In the course of evolution, these receptors have become "parasitized" by lethal agents such as bacteriophage, bacteriocins ("killer proteins"), and antibiotics. One of the earliest genetic lesions studied in *E. coli*, *tonA* ("T-one"), now *fhuA*, was that specifying ability to attach phage T1. This pore turned out to be the receptor for ferrichrome, although it also enables transport of several phages, colicin M, and albomycin. Similarly, the receptor for ferric enterobactin, FepA, is the site of penetration of colicins B and D, and the bacteriocin colicin utilizes the ferric aerobactin receptor. This is the general pattern with siderophore receptors; namely, they also act as receptors for a variety of lethal agents. Thus, the receptor for an uncharacterized siderophore of *Yersinia enterocolitica* has been shown to serve as receptor for pesticin.

A second classical gene in *E. coli* coding for the TonB protein, required for phage infection and for iron supply via the many siderophore and inorganic iron uptake systems of the bacterium has also been studied. TonB, located in the cytoplasmic membrane, was viewed as providing some kind of link to the outer membrane, but clarification, in molecular terms, had to await sequencing of the genes for the receptors. In an elegant experiment, the deletion of a particular loop converted FepA into a nonspecific diffusion channel. A similar finding was reported for ferrichrome transport in FhuA. Apparently, these bacteria have evolved a sophisticated mode of active iron transport in which the energy of the cytoplasmic membrane has been linked to the outer membrane siderophore receptors (Davidson and Nikaido 1991).

Applications of siderophore/siderophore producing microbes.

Siderophores are biological molecules having diverse Agricultural, Clinical and Environmental applications.

In agriculture

These chemical compounds produced by microorganisms in the rhizosphere can increase the availability and uptake of iron. It has been demonstrated that plants are able to use the hydroxamate-type siderophores ferrichrome, rodotorulic acid and ferrioxamine B; the catechol-type siderophores, agrobactin; and the mixed ligand catechol-hydroxamate-hydroxy acid siderophores biosynthesized by saprophytic root-colonizing bacteria. All of these compounds are produced by rhizospheric bacterial strains, which have simple nutritional requirements, and are found in nature in soils, foliage, fresh water, sediments, and seawater (Klopper et al., 1980).

Fluorescent pseudomonads have been recognized as biocontrol agents against certain soil-borne plant pathogens. They produce yellow-green pigments (pyoverdines) which fluoresce under UV light and function as siderophores that deprive pathogens of the iron required for their growth and pathogenesis. Fluorescent pseudomonads form a line of siderophores comprised of a quinoline moiety, responsible for the fluorescence, and a peptide chain of variable length bearing hydroxamic acid and α -hydroxy acid functions. Capacity to form these pseudobactin or pyoverdine type siderophores has been associated with improved plant growth either through a direct effect on the plant, through control of noxious organisms in the soil, or via some other route. Pyoverdine siderophores produced by Pseudomonads have shown to control the wilt diseases of potato caused by *Fusarium oxysporum* (Schippers et al. 1987, They are also active against *Gaeumannomyces graminis* which is associated with a deficiency of wheat and barley growth (Voisard et al. 1989 Siderophores produced by *Bacillus subtilis* also play a pivotal role in the biocontrol of *F. oxysporum*, which is responsible for the Fusarium wilt of pepper (Yu et al. 2011).

Nitrogenase can be said to be an iron-intensive enzyme complex and the symbiotic variety, as found in *Rhizobium* spp., may require an intact siderophore system for expression of this exclusively prokaryotic catalyst upon which all life depends. (Powel et al., 1980).

In therapy As naturally occurring chelating agents for iron, siderophores might be expected to be

somewhat less noxious for deferrization of patients suffering from transfusion-induced siderosis. A siderophore from *Streptomyces pilosus*, desferrioxamine B, is marketed as the mesylate salt under the trade name Desferal and is advocated for removal of excess iron resulting from the supportive therapy for thalassemia. (Hershko et al., 2002).

The potency of common antibiotics has been elevated by building into the molecules the iron-binding functional groups of siderophores. The objective here is to take advantage of the high affinity, siderophore-mediated iron uptake system of the bacteria. Siderophores have applications in medicine for iron and aluminum overload therapy and antibiotics for improved targeting. Understanding the mechanistic pathways of siderophores has led to opportunities for designing small-molecule inhibitors that block siderophore biosynthesis and therefore bacterial growth and virulence in iron-limiting environments (Ackrill et al., 1980).

Siderophores are useful as drugs in facilitating iron mobilization in humans, especially in the treatment of iron diseases, due to their high affinity for iron. One potentially powerful application is to use the iron transport abilities of siderophores to carry drugs into cells by preparation of conjugates between siderophores and antimicrobial agents. Because microbes recognize and utilize only certain siderophores, such conjugates are anticipated to have selective antimicrobial activity (Benz et al., 1982).

Microbial iron transport (siderophore)-mediated drug delivery makes use of the recognition of siderophores as iron delivery agents in order to have the microbe assimilate siderophore conjugates with attached drugs. Siderophore-antibiotic conjugates – of the type Sideromycins can be used for selective delivery of antibiotics in antibiotic resistant bacteria causing serious illnesses. These drugs are lethal to the microbe and cause the microbe to apoptosise when it assimilates the siderophore conjugate. Through the addition of the iron-binding functional groups of siderophores into antibiotics, their potency has been greatly increased. This is due to the siderophore-mediated iron uptake system of the bacteria. Several natural siderophore-antibiotic conjugates are Albomycins, ferrimycins, danomycins, salmycins (isolated from *Streptomyces* and *Actinomyces*), microcins (isolated from enteric bacteria). Synthetic Siderophore-antibiotic conjugates-can be prepared by binding potent antibiotics such as beta lactam (Carbacephalosporins), erythromycin, sulphonamides, spiramycin, vancomycin, nalidixic acid, norfloxacin, antifungals such as 5- flourocytosine and

neoenactins. Miethke and Marahiel 2007, Budzikiewicz 2001, and Diarra et al 1996..

In Environment

Siderophores are potent to resolve various environmental problems like heavy metal accumulation, rust removal, biofouling, dye degradation, sewage treatment and bioleaching, etc 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 Cd, Cr, Cu, Hg, Pb and Ni 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 be used to treat radioactive waste prior to storage or to decontaminate soils and water (Ruggiero et al., 2000; VonGunten and Benes, 1995).

Ecological point of view also several reports also show that siderophore producing bacteria favour the growth of some unculturable bacteria when grown in a coculture. Kaeberlein et al. 2002., Lewis et al. 2010). Studies also showed that addition of siderophore exogenously enhanced the growth of uncultivable marine bacterial species in laboratory medium. (Guan and Kamino 2001). Thus this approach can help in facilitating the growth and cultivation of unculturable organisms. (D'Onofrio et al. 2010). In this way the potential applications of such unculturable organisms can be studied.

Siderophores and Virulence

A role for iron in the virulence mechanism of several microbes attacking man and other animals is well established. An adequate iron supply for many pathogenic species is critical since transferrin has a very high affinity for the metal and the protein is normally only about one-third saturated with iron. Strains of *E. coli* causing disseminating infection were found to harbor ColV plasmids carrying the aerobactin synthesis and transport genes. The siderophore system of *Y. enterocolitica* is correlated with the virulence of the organism. Regarding phytopathogens, it should be recalled that the virulence-associated iron chrysoactin uptake apparatus of *Erwinia chrysanthemi* involves an operon encoding transport and biosynthetic functions (Litwin and Canderwood, 1993, Wooldridge and Williams, 1993).

Conclusion

Iron is an important element for all living cells but due to its poor solubility its uptake by cells is difficult. For the iron supply of living cells, some microorganisms include bacteria, fungi, actinomycetes and algae produce an iron chelator, siderophores, outside the cells and the siderophore is chelated with ferric iron. Microbial siderophores have enormous scope and applications for the sustainability of humans, animals and plants. Currently the applications of siderophores in clinical, agricultural, ecological and environmental sector are reported to some extent. But still siderophore research is not that developed in microbiology research laboratories. Further research on siderophores is to be carried out to unravel its properties and applications to use them for the betterment of the society and the environment.

References

1. 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.
2. Albrecht-Gary A.M. & Crumbliss A.L., 1988 Metal ions in biological systems. Handbook of microbial iron chelates, CRL Press New York, 1988.
3. Albrecht-Gary A.M. & Crumbliss A.L., 1998, Coordination chemistry of siderophores, Thermodynamics of iron chelation and release. Metal ions in biological systems. Vol.32, 239-327.
4. 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.
5. Boukhalifa H., Reilly S.D., Michalczyk R., Iyer S. & Neu M.P. 2006. Iron(III) coordination properties of a pyoverdine siderophore produced by *Pseudomonas putida* ATCC 33015. *Inorganic Chemistry*, 45, 5607-5616.
6. Budzikiewicz H. Siderophore-Antibiotic Conjugates Used as Trojan Horses Against *Pseudomonas aeruginosa*. 2001. *Curr Top Med. Chem.* May;1(1):73-82.
7. Chaudhari B L and Chincholkar S B 1998, Microbial siderophores In Selected topics in Biotechnology ed. A.M Deshmukh, Pama Publications, Karad, India. pp 32-42.

8. D'Onofrio A, Crawford JM, Stewart EJ, Witt K, Gavrish E, Epstein S, Clardy J, Lewis K (2010) Siderophores from neighboring organisms promote the growth of uncultured bacteria. *Chem Biol* 17:254–264.
9. 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.
10. Dhungana S., Ratledge C. & Crumbliss A.L.. 2004. Iron chelation properties of an extracellular siderophore exochelin MS. *Inorganic Chemistry*, 43, 6274–6283.
11. Diarra MS, Lavoie MC, Jacques M, Darwish I, Dolence EK, Ghosh AJA, Miller MJ and Malouin F. Species selectivity of new siderophore – drug conjugates that use specific iron uptake for entry into bacteria. *Antimicrob Agents Chemother* 1996;40:2610-7.
12. Emery T. Isolation, Characterization, and Properties of Fusarinine, a δ -Hydroxamic Acid Derivative of Ornithine*1965 *Biochemistry*, 4 (7), pp 1410–417. DOI: 10.1021/bi00883a028
13. Gibson F and Magrath D I , *Biochemica and Biophysica Acta* 192- 1969
14. Glick B.R., C.L. Patten, G. Holguin, G.M. Penrose *Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria* Imperial College Press, London (1999).
15. Guan LL, Kamino K (2001) Bacterial response to siderophore and quorum-sensing chemical signals in the seawater microbial community. *BMC Microbiol* 1(1):27
16. Hershko, C., Link, G., and Konijn, A. M.2002. Cardio protective Effect of Iron Chelators, in *Iron Chelation Therapy* ,ed. Hershko, C., ed, Kluwer Academic / Plenum Publishers, New York, Vol. 509, 1Ed pp. 77-89
17. Jalal, M. A. F., Mocharia, R., Barnes, C. L., Hossain, M. B., Powell, D. G., Eng-Wilmot, D. L., Grayson, S. L., Benson, B. A., and Van der Helm, D., 1984, Extracellular siderophores from *Aspergillus ochraceous*, *J. Bacteriol.* **158**:683–688.
18. Jalal M A F and Van der Helm D 1991. Isolation and spectroscopic identification of fungal siderophores. In. *Handbook of Microbial iron chelates*, ed. G Winklemann, CRC Press, Boca Raton, USA. pp-235-269
19. Kaeberlein T, Lewis K, Epstein SS (2002) Isolating “uncultivable” microorganisms in pure culture in a simulated natural environment. *Science* 296:1127–1129.
20. Kloepper, J.W., Leong, J., Teinize, M and Schroth, M.N. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature.* 286, 885-886.
21. Kraemer S.M.2004, Iron oxide dissolution and solubility in the presence of siderophores. *Aquat Sci*, 66,3-18.
22. Leong, S.A. and Neilands, J.B. 1982. Siderophore production by phytopathogenic microbial species. *Arch. Biochem. Biophys.* 281, 351-359.
23. Lewin, 1984. How microorganism transport Iron. *Science.* 225, 401-402.
24. Lewis K, Epstein S, D'Onofrio A, Ling LL (2010) Uncultured microorganisms as a source of secondary metabolites. *J Antibiot* 63(8):468–476.
25. Litwin, C. M., and Calderwood, S. B. 1993. Role of iron in the regulation of virulence genes. *Clin. Microbiol. Rev.* 6, 137-149.
26. 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.
27. 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.
28. Menon B A 2005, Detection of siderophores by thinlayer Chromatography *Jour. Chem. Soc. Pak*, Vol.27, No.6 658-661
29. Messenger AJM & Ratledge C. Siderophores , In Moo-Yong M (ed) 1985 *Comprehensive Biotechnology: the principles, applications and regulations in Biotechnology in industry and agriculture and medicine*, Vol.3 Pergamon New York, pp-275-295.
30. Miethke M and Marahiel M A. Siderophore-Based Iron Acquisition and Pathogen Control. *Microbiol Mol Biol Rev* 2007;71(3):413–51.

31. Powell, P.E., Cline, G.R., Reid, C.P.P and Szanislo, P.J. 1980.Occurrence of hydroxamate siderophore iron chelators in soils. *Nature*. 287, 833-834.
32. Rane M. R, Naphade B. S, Sayyed R Z and Chincholkar S B (2005) Methods or Microbial iron Chelator (Siderophore) Analysis. In *Basic Research and Applications of Mycorrhizae* Ed. G. K. Podile and A.Verma. I. K. International Pvt. Ltd.
33. Renshaw J.C., Robson JD, Trinci APJ,Weibe MG, Livens F, Collison D,Taylor RJ 2002, Fungal siderophores, Structures, Functions and Applications. *Mycol Res*, 106,1123-1142.
34. Ruggiero, C. E., M. P. Neu, J. H. Matonic, andS. D. Reilly. 2000. Interactions of Pu with desferrioxamine siderophores can affect bioavailability and mobility. *Actinide Res.Q.* 2000, 16-18.*Top Med Chem* 2001;1:73-82.
35. Von Gunten, H. R. and Benes, P. 1995 Speciation of radionuclides in the environment. *Radiochim. Acta.* 69, 1-29.
36. Wandersman, C. and Delepelaire, P. (2004) Bacterial Iron Sources: From Siderophores to Hemophores. *Annual Review of Microbiology*, 58, 611-647. <http://dx.doi.org/10.1146/annurev.micro.58.030603.123811>
37. Wandersman, C. and Delepelaire, P. (2004) Bacterial Iron Sources: From Siderophores to Hemophores. *Annual Review of Microbiology*, 58, 611-647. <http://dx.doi.org/10.1146/annurev.micro.58.030603.123811>
38. Wooldridge, K.G., and Williams, P.H. 1993. Iron uptake mechanism of pathogenic bacteria. *FEMS Microbiol. Rev.* 12, 325-348.
39. Yang Huiming, Wipada Chaowagul, Pamela A.Sokol, Siderophore Production by *Pseudomonas pseudomallei*. 1991 Vol.59, No.3 P.776-780.
40. Yu X, Ai C, Xin L, Zhou G (2011) The siderophore producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. *Eur J Soil Biol* 47:138-145

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