

# 17. Bacterial Siderophores and their Biotechnological applications

C. Mohandass

Biological Oceanography Division  
National Institute of Oceanography  
Dona-paula, Goa.403 004.India.

## Introduction

Siderophore is the Greek phrase for “iron bearer” and is applied to molecules that can bind metals at very high affinities. Marine bacteria as do their terrestrial counterparts, secrete siderophores, which are small peptide molecules, readily assembled by short dedicated metabolic pathways which contain side chains and functional groups that can provide a high affinity set of ligands for coordination of ferric ions. In the marine environment bacterial growth depends in part upon the availability of iron, which is an essential requirement for most microorganisms. Although heterotrophic bacteria require conditions of up to one micro molar iron for growth, the total amount of iron in surface ocean water is subnanomolar. This limiting amount of iron has implications in the biogeochemical cycling of carbon and in limiting phytoplankton growth. Marine bacteria can successfully compete for this limited nutrient using a specialized iron transport system, including the production and release of siderophores, which mediate iron transport into the cell by specific membrane receptor proteins and transport systems (Lewin, 1984; Messenger and Ratledge, 1985; Reid and Butler, 1991)

Microorganisms allow iron into the cell in dissolved form. This high affinity system has two parts; the production of an iron binding molecule, which is excreted from the cell and the elaboration of a membrane receptor molecule, which binds the iron complex and transport the metal to the cell's interior. Membrane receptor and enzymes that synthesize the iron binding molecules are coded by five genes in one operon, which is turned off when sufficient iron has been taken into the cell (Lewin, 1984).

Siderophores are novel structures, many containing modified amino acids not found elsewhere in nature. Great variation is seen in siderophore structure from one species to another. There are three main kinds of iron containing functional groups known as hydroxamate, catecholate and carboxalate siderophores.

## Hydroxamate siderophores

Hydroxamate siderophores are produced by bacteria and fungi. Most hydroxamate siderophores contain three secondary 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<sup>3+</sup>. 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) ( Fig 1). The hydroxamate siderophores was detected by Neiland's spectrophotometric assay (1981) or by tetrazolium salt test (Snow, 1954).

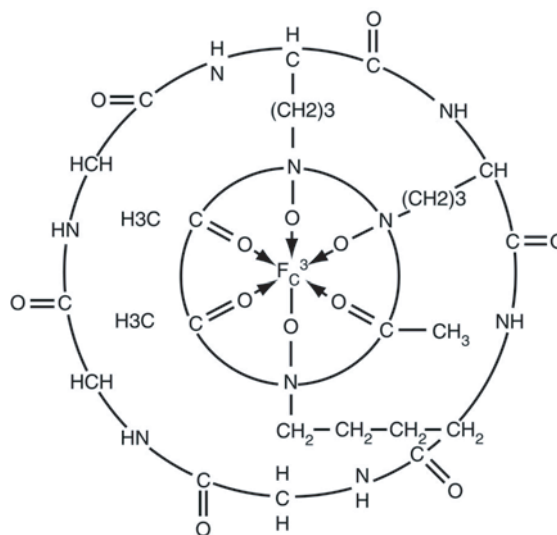


Fig 1. Hydroxamate siderophore ferrichrome from *Ustilago sphaerogena*. (Source: Messenger and Ratledge 1985)

## Catecholate (Phenolates) siderophores

These are sometimes referred to as phenolates. These are produced only by certain bacteria. Each catecholate group provides two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed as in the case of hydroxamate siderophores (Fig. 2). The catecholate nature of the siderophore is also detected by Neilands spectrophotometric assay (Neilands, 1981), Formation of wine coloured complex with  $\text{FeCl}_3$  that absorbs maximally at 495 nm, Indicates catecholate nature of siderophores. This can be confirmed with Arnows test (Arnaw, 1937), using nitrite molybdate reagent.

## Carboxylate (complexones) siderophores

Carboxylates (earlier called complexones), produced by bacteria (certain *Rhizobium* and *Staphylococcus* strains) and fungi belonging exclusively to Mucorales, coordinate iron with carboxyl and hydroxyl groups (Dave and Dube, 2000). Carboxylate nature of siderophore was detected by Vogels chemical test (Vogel, 1987). It is further confirmed by spectrophotometric assay (Shenker et al., 1992).

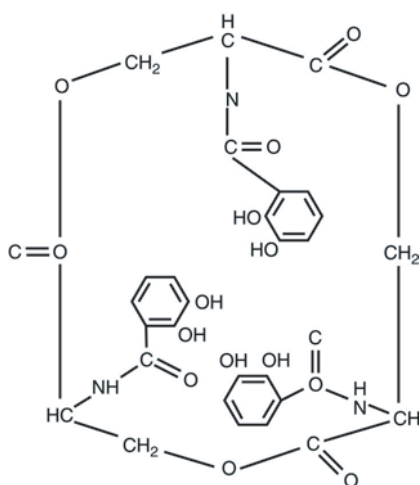


Fig 2. Catecholate siderophore Enterochelin produced by *E. coli*. (Source: Messenger and Ratledge 1985.)

## Research on siderophores

Siderophore synthesis, their structure, properties and uses have been studied from many terrestrial microorganisms (Teintze et al., 1981; Smith et al., 1985; Matzanke et al., 1989; Calugay et al., 2002; Crosa et al., 2002; Table 1). Surprisingly, very little is known about the nature of the siderophores produced

by marine microorganisms. Structure of anguibactin produced by a fish pathogen, *Vibrio anguillarum* (Jalal et al., 1989) and bisucabarin produced by a deep-sea bacterium *Alteromonas haloplanktis* (Takashashi et al., 1987), are known (Fig 3.) Strains of open ocean bacteria from the genera *Vibrio*, *Alteromonas*, *Alcaligenes*, *Pseudomonas* and *Photobacterium* have been surveyed for their ability to produce siderophores (Trick 1989; Goynes and Carpenter, 1974). Bechet and Blondeau (1998) studied the iron deficiency-induced tetracycline production in submerged cultures by *Streptomyces aureofaciens*. Cells were cultured at 30° C with 100 rpm shaking in a defined medium. Tetracycline biosynthesis was inhibited either by free iron at concentrations above 1-2  $\mu\text{mol l}^{-1}$ , or by chelated iron provided by siderophores of this bacterial strain. Late static iron-containing cultures allowed cell differentiation and sporulation and led to tetracycline synthesis. Duffy and Defago (1999) studied the environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens*. White et al., (1995) studied the role of microorganisms in biosorption of toxic metals and radionuclides. Metabolism-dependent mechanisms of metal removal in living microorganisms include metal precipitation as sulfides, complexation by siderophores and other metabolites, sequestration by metal binding proteins and peptides, transport and intracellular compartmentation. New antiparasitic siderophores and their substituted derivatives were obtained from *Klebsiella pneumoniae* culture (Gysin et al., 1991). The active fractions may be formulated into new anti parasitic compositions. This action,

### Structure of two marine bacterial siderophores

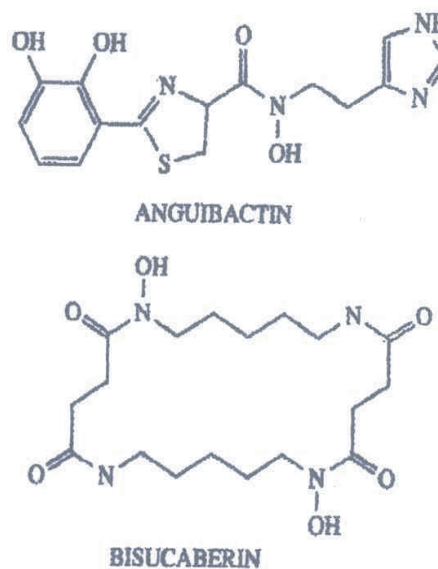


Fig 3. Anguibactin produced by fish pathogen *Vibrio anguillarum* and Bisucabarin produced by deep-sea mud bacterium *Alteromonas haloplanktis*. (Source: Reid and Butler. 1991)

designated 'plasmocidine activity', is specific and is not reversed by iron. The new siderophores act through a mechanism that is different from that of other antimalarial agents and is non-toxic (Gysin et. al., 1991).

Yeole et al., (2001), examined the extra cellular siderophore production by a *Pseudomonas* sp in different media under iron deficient condition. Dave and Dube (2000) studied regulation of siderophore production by Fe (III) in certain fungi and fluorescent *Pseudomonas*. Boopathi and Rao (1999) studied the siderophore from *Pseudomonas putida*. Cultivation was performed on minimal succinic acid medium at 28° C and pH 7.2. The purified compound exhibited siderophore activity for *P. putida* and fungicidal activity on the phytopathogens. Fungicidal activity of the compound may be useful in the control of wild disease in chickpea and leaf spot on rice, either by introducing the *P. putida* directly or by applying the siderophore into the rhizosphere. Patil et al., (1999) studied the siderophores of the fungus *Cunninghamella blates/eeana* that is recognized industrially for progesterone biotransformation. This fungus produced two siderophores at low concentrations of iron (up to 40 µM iron in the growth medium).

Suneja et al., (1994) optimized the culture conditions of *Azotobacter chroococcum* (a plant growth hormone and fungicide producing bacterium which may be used as a biofertilizer) and these were tested for hydroxamic acid production in iron-limited modified Burk medium (BM). Maximum hydroxamic acid production was

observed in soil isolate 111 followed by an analog-resistant mutant Mac 68. Experiments on production of catecholate type of siderophore by *Azospirillum lipoferum* were carried out by Shah et al., (1992 & 1993) by optimizing conditions for siderophore production using different carbon (1g l<sup>-1</sup>) and nitrogen sources (10 mM) and K<sub>2</sub>HPO<sub>4</sub> concentrations (0.1-1.0 g l<sup>-1</sup>). Siderophore production was maximum when malic acid, succinic acid and gluconic acid were used as C-source, and was completely inhibited when citric acid was used. Of the N-sources tested, ammonium chloride supported maximum siderophore production and growth of *A. lipoferum*. The optimal concentration of K<sub>2</sub>HPO<sub>4</sub> was 0.5 g l<sup>-1</sup>. A high aeration rate suppressed siderophore production. Maximum production occurred when a 250 ml flask containing 175 or 200 ml of medium was used.

## Siderophore production by marine organisms

We have isolated a marine bacterium, which could remove ink from inkjet-printed paper pulp (Raghukumar, et al., 2002). As the ink used in inkjet printers contains iron oxide, we hypothesized that siderophores produced by the bacterium are involved in the deinking process. Although we could not prove this conclusively, we further isolated few marine bacterial strains that were producing siderophores (Fig. 4) and some of them were effective in deinking inkjet printed-paper pulp. However, not all siderophore-producing bacteria could remove ink from the printed-paper pulp. These results encouraged us to do a thorough screening and understand the mechanism of siderophore production and action by marine bacteria and find newer applications of siderophore-producing organisms.

Marine phytoplankton play an important role on the carbon cycle in the oceans. However, growth of phytoplankton is often limited by shortage of iron in the oceans. Iron limitation in the oceans decreases the efficiency of the biological carbon pump. Phytoplankton must have developed a sophisticated mechanism to uptake iron. However, little is known about the uptake mechanism. Given the importance of the biological pump in controlling atmospheric CO<sub>2</sub>, elucidating the iron uptake mechanism of phytoplankton under the iron deficient conditions is significantly important. Bacteria associated with Phytoplankton might secrete siderophores in a similar manner. Siderophore production by eukaryotic phytoplankton has not been reported.

Table 1. Examples of siderophores produced by microorganisms

Organisms	Siderophores
<i>Ustilago sphaerogena</i>	Ferrichrome
<i>Streptomyces pilosus</i>	Desferrioxamines
<i>Escherichia coli</i>	Enterobactin
<i>Aeromonas hydrophila</i>	Amonabactin
<i>Aerobacter aerogens</i> , <i>Salmonella</i> sp, <i>Klebsiella pneumoniae</i>	Aerobactin
<i>Vibrio cholerae</i> <i>Vibrio anguillarum</i>	Vibriobactin (Marine) Anguibactin
<i>Acinetobacter calcoaceticus</i>	Acinetobactin
<i>Mycobacterium tuberculosis</i>	Mycobactin
<i>Pseudomonas aeruginosa</i>	Pyoverdin and Pyochelin
<i>Yersinia pestis</i>	Yersiniabactin
<i>Alteromonas haloplanktis</i>	Bisucabarin (Marine)

## Biotechnological applications

The importance of these siderophores extends beyond their immediate role in microbial physiology and their applications in biotechnology (Messenger and Ratledge, 1985). Siderophores and their substituted derivatives have a lot of applications. For example, haemochromatosis is a disorder wherein there is a progressive increase in body iron content causing iron deposits in liver, heart or pancreas. Primary haemochromatosis is caused by increased absorption of iron from the gastrointestinal tract without any associated anemia. Desferrioxamine in the form of Desferal Siderophore is used in the treatment of haemochromatosis.

## Sideromycins

Sideromycins are iron-chelating antibiotics produced by streptomycetes. Albomycin and ferrimycin are two examples of this group being closely related to



Fig. 4. The yellow orange hallow surrounding the bacterial colony is indicative of the production of an Fe-binding compound such as siderophore, which removes Fe (III) from the Fe (III)–CAS–HDTMA complex in the plate and turns the blue dye to yellow color.

ferrichrome. Both antibiotics contain an extra chemical group attached to the basic siderophore structure. Thus, they use the siderophore transport system to gain access to the cell, but once inside the cell their mechanism of action is not against iron transport or its acquisition. Instead these antibiotics exert their effect by inhibiting protein synthesis, similar to many other antibiotics. When the functional group is removed, the molecules lose their antibiotic activity but their growth promoting activities as siderophores are retained (Snow, 1970). There are of course, other antibiotics that probably fall in to this category but these two are probably the best understood examples.

## Biomedical application of siderophores

The development of electricity generation by nuclear energy has led to increased human exposure to transuranic elements such as aluminum. Investigations have been carried out to evaluate the capacity of siderophores in removing such elements from the body. Administration of desferrioxamine lowers the level of aluminum in the body and relieves the symptoms of the disease (Ackrill et al., 1980; Arze et al., 1981; Pogglitsch et al., 1981). Desferrioxamine has also been used to remove vanadium. In rats Desferal reduced the vanadium content in kidney by 20%, in lung by 25% and in liver by 26% when administered at  $100 \mu\text{mol kg}^{-1}$  following a dose of  $5 \mu\text{mole kg}^{-1}$  of  $\text{Na}^{48}\text{VO}_3$ . Both urinary and faecal excretion increased at this dose (Hansen et al., 1982). Desferrioxamine has proved successful in the treatment of dialysis encephalopathy, which is a major complication of long-term dialysis. It is caused by the accumulation of aluminum in the brain from the dialysis water supply.

Siderophore from *Klebsiella pneumoniae* has been used as an antimalarial agent (Gysin et al., 1991), and in cosmetics as deodorants (Johnson et al., 2003).

To date, more than 500 siderophores produced by bacteria, yeast and fungi have been identified (Ratledge and Dover 2000). Human body usually contains 3-4 g iron, 70% of which is in hemoglobin and mycoglobin and the rest in intracellular enzymes and storage iron. Ferritin, an iron storage protein containing up to 4500 Fe atoms per molecule provides the major non-toxic store of iron in the liver, spleen and other organs, which can be mobilized when needed.

## Agricultural uses of siderophores

In agriculture, inoculation of soil with *Pseudomonas putida*, which produces pseudobactin, increases growth and yield of various plants. (Kloepper et al., 1980). Electricity generation by nuclear energy has resulted in human exposure to transuranic elements like aluminum. In view of this, investigations have been carried out to assess the capability of siderophores to remove such elements from the body. (Ackrill et al., 1980, Arze et al., 1981; Pogglitsch et al., 1981).

Powell et al., (1980) suggested that hydroxamate siderophores are present in soil at concentrations high enough ( $10^7$  to  $10^8$ ) to be taken up by plant roots. In many ways, iron is involved in nitrogen fixing by soil bacteria. Recently, an iron and molybdenum cofactor for nitrogenase has been discovered (Messenger and Ratledge, 1985). Since siderophores such as

mycobactin can bind Mo as well as Fe, it is possible that the organisms deliberately produce siderophores capable of binding both the metals. Siderophore production, however is not just restricted to microorganisms grown in laboratories. Powell et al., (1980) have shown that hydroxamate siderophores are present in various soils and they are also produced in aquatic environments. Importance of siderophores in maintaining the balance in mixed microbial population in the soil was realized back in 1950's itself. Burton et al., (1954) had shown that some microbes synthesize siderophores whilst others use them without synthesizing any.

## Future prospects

Our knowledge of the structures of various siderophores and the presence of microbial membrane receptors operating in iron uptake from these iron siderophore complexes has opened newer avenues for research. The search for antibiotics similar in structure to the siderophores and disrupting their normal functioning is now possible. Research to find out more effective and preferably cheaper chelating agents than desferrioxamine for use in chelation therapy will no doubt continue. Our knowledge of the structure and mode of action of many siderophores should aid in future research to design of such chelating agents. In the marine environment, the role of siderophores in primary production, their qualitative and quantitative variations, structural and functional properties in relation to cumulative production of various nutrients, ocean biomasses are to be vigorously investigated.

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