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Microbial Siderophore and Their Application: A Review

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ABSTRACT
Iron is one of the major limiting factors and essential nutrients of microbial life. Since in nature it is not readily available in the preferred form, microorganisms produce small high affinity chelating molecules called siderophores for its acquisition. 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. Siderophores also play a critical role in the expression of virulence and development of biofilms by different microbes. Siderophore and their derivative have large application in agriculture as to increase soil fertility and biocontrol for bacterial and fungal pathogen. This review is intended to provide a general overview on siderophore along with its role and applications.

Introduction

Siderophores (from the Greek: “iron carriers”) are defined as relatively low molecular weight, ferric iron specific chelating agents elaborated by bacteria and fungi growing under low iron stress [57]. Iron is an essential element which is difficult to obtain due to its low solubility under the physiological conditions, and it is also the most important transition metal iron for nearly all living systems. The role of this compound is to scavenge iron from the environment and to make the mineral, which is practically always essential, existing to the microbial cell.Siderophores are among the strongest soluble Fe^{3+}binding agent known[54]. Also it has been reported that siderophores are low molecularweightFe^{3-} specific compounds and most of them contain either hydroxamates or catechloes as the iron- sequestering groups. Its molecular weight is low (500-1000 Da). It is a ferric-specific ligand; its biosynthesis is regulated by iron levels in the medium and the formation constant is around 1030 or higher. The siderophores chelate iron in the extracellular environment and the resulting ferric siderophore complex is recognized by research in this field which has begun about five decades ago, and interest in it has accrued with the realization that most aerobic and facultative anaerobic microorganisms synthesizes at least one siderophore. Siderophores have been related to virulence mechanisms in microorganisms pathogenic to both animals and plants. The production of siderophore can be detected by several methods including absorption spectra,bioassay or blue CAS complex thus causing its colour change to orange. Different siderophore assays have been recommended for evaluation of siderophore portion. This isparticularly being important when evaluating the potential of a strain for biocontrol [46,71,51,16]. In addition, they have clinical applications and are conceivably important in agriculture.

Iron is a vital element required by all living organisms for various cellular processes such as eletron transport c44whain and as a cofactor for many enzymes [41].Microorganisms growing under aerobic condition need iron for varieties of functions which includes reduction of oxygen for the synthesis of ATP, formation of hemo and also for other important purposes. The aerobic atmosphere of the planet has caused the surface iron to oxidize and induce the level of free iron. Therefore, a way for iron acquisition by producing iron chelating molecule i.e. siderophore. Siderophores are low molecular weight(<10 KD) iron chelating compounds synthesized by many bacteria such as *Pseudomonas*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Serratia*, *Azospirillum* and *Rhizobium*is adopted by microorganisms[27,42], siderophore from 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 necessaryironhas been taken into the cell [40]. Some bacteria produce one or more siderophore which can be utilized by other microorganisms for iron and other metals acquisition. This property of siderophore increased their application, also siderophore have been related to virulence mechanisms in microorganisms pathogenic to both animals and plants. In addition, they have application in clinical, agriculture and environmental fields. At present-day nearly 500 siderophores are reported from selected microorganisms. A great dissimilarity is seen in siderophore structure from one species to another. There are three main kings of siderophore known as hydroxamate, catecholate and carboxalate.Fermentative production of siderophore is tricky due to its dependence oncritical amounts of iron. Moreover, several factors influence the production ofsiderophore. The present review is directed to elaborate this issue based on experimentsperformed in our laboratory.

Structure of siderophore

There are many different types of siderophore available and generally be classified into two structural groups, hydroxamates and catecholate compounds. Despite their structural differences all from an octahedral complex with six binding coordinates for Fe^{3+}. Siderophores can be detected in bacterial cultures using chemical assays, bioassays and function assay. There are specific for hydroxamates and

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catecholate groups [53].For all siderophore do not have the appropriate function groups. Bioassay use selected strains of bacteria of siderophores for growth in a low iron medium [51]. Culture or extracellular fluids of siderophore producing bacteria can be promote growth of these strains, however do not detect all siderophores. The chrome azurol S assay is a functional assay that is often to detect siderophores. Chrome azurol S is a blue compound that binds iron but changes to orange colour azurol S assay is independent of siderophores. The relatively easy to detect siderophore in many microorganisms [16].

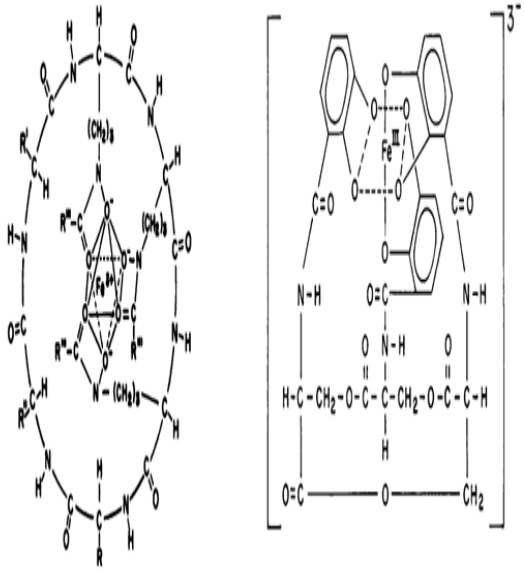


Fig 1. General structure of the ferrichromes, prototypical hydroxamate Types of siderophore.
Fig 2. Ferric enterobactin, a prototypical catechol type siderophore

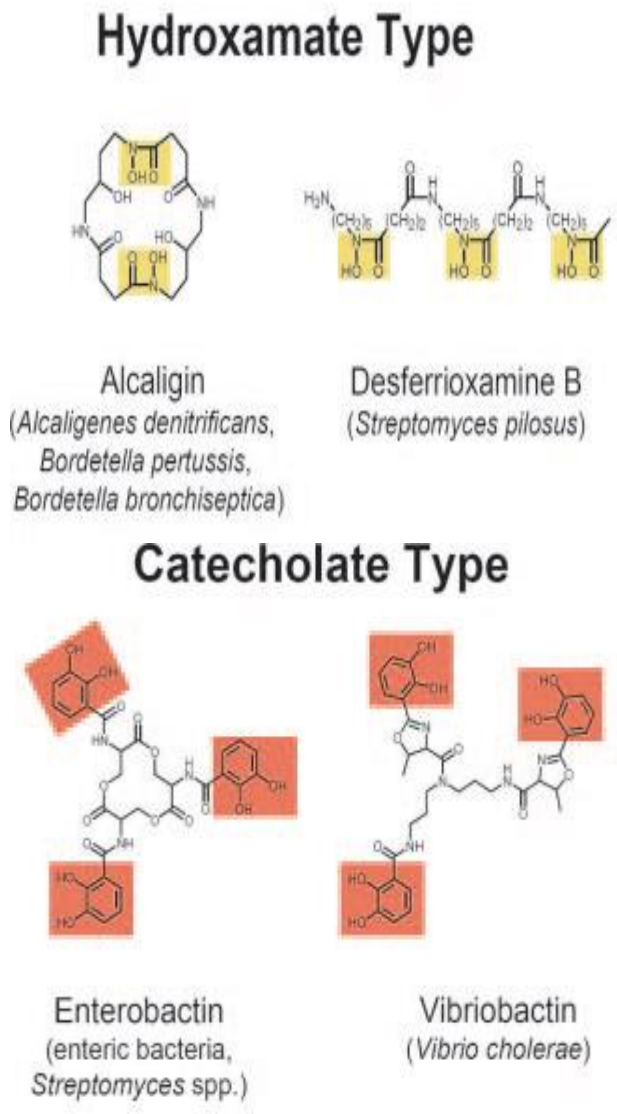
Type Of Siderophore:

Hydroxamate siderophore

Hydroxamate siderophores are produced by bacteria and fungi. Most hydroxamate group, C(=O)N-(OH) R, where R is an amino acid or a derivative. Each hydroxamate group provides two oxygen molecules, which from a bidentate ligand with iron. Therefore, each siderophore forms a hexadentate octahedral complex with Fe^{3+}. Hydroxamate siderophore 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 [49]. Ferribactin produced by *Pseudomonas fluorescens* is known to be a hydroxamate. Gonobactin and nocobactinproduced in small quantities by *N. gonorrhoeae* and *N.meningitids* are also hydroxamates. The hydroxamate siderophore was detected by Neiland's spectrophotometric assay [58].

Catecholate siderophore

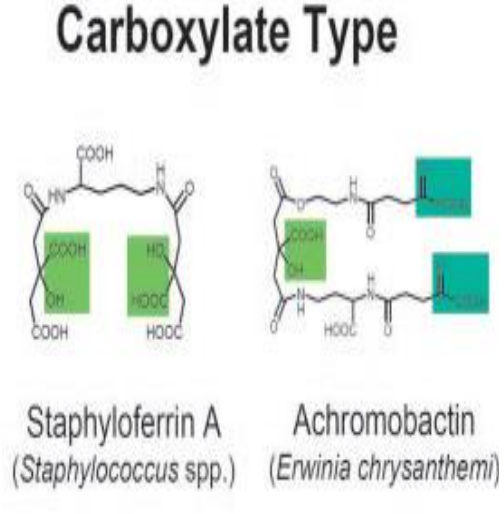
Enterochelin the cyclic trimester of 2, 3-dihydroxybenzoylserine, is produced by *E-coli, S.typhimurium* and *K. pneumonia* and is the prototype of the catecholate siderophore. Each catecholate group provides two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed as in the case of the hydroxamate siderophores. Liner catecholate siderophore are also produced in certain species. Agrobactin and parabactin are produced by Agrobacterium tumefaciens and Paracoccus *denitrificans respectively*. *Erwinia carotovora* produced catecholated while *Pseudomonas* produced a mixed catecholate-hydroxamate siderophore [39]. The catecholate nature of the siderophore is also detected by Neilands spectrophotometric assay [58]. Formation of wine colour red complex with $FeCl_3$that absorbs maximally at 495 nm indicates catecholate nature of siderophores.



Carboxylate siderophore

The universal assay for siderophore detected [71], has facilitated the detection of siderophore that are neither catecholates nor hydroxamates. The best characterized carboxymates type siderophore with a novel structure is *rhizobactin. Rhizobactin* is produced by *Rhizobium* meliloti strain DM4 and is an amino poly (carboxylic acid) with ethylenediaminedicarboxyl and hydroxycarboxyl moieties as iron chelating groups. *Staphylococcus hyieus* DSM20459, is another member of this class of complex on siderophore satpholoferric and consists of one acid residues linked by two amide bonds. Siderophore-mediated iron uptake in microorganisms is both a receptor and an energy-dependent process [74]. Such systems have been well studied in *Escherichia coli* [84]. Siderophores are part of a multi-component system for transporting ferric iron into a cells other components include a specific outer membrane receptor protein Fec A,Fec A and TonB-ExbB-ExbD protein complex in the inner membrane,a periplasmic binding protein, and an inner membrane ATP dependent Fep CDE protein. 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 to the cell via Fec A and Fep A outer membrance receptor (OM).Later siderophore iron complex release in cytoplasm with the help of membrane protein. 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 molecular or the reduction of $Fe^{3+}$by a NAD (P) H- linked siderophore reductase or Ent A,B,C,D protein. The resulting Fe^{3+}doesn't have a high affinity for siderophore and therefore dissociated from the complex.



Biosynthesis

The siderophore for which we have the greatest inventory of information with regard to its anabolism is aerobactin first isolated from *Aerobacter* aerogenes [26]. Subsequently, it was detected as a product of pColV-K30, a plasmid commonly borne by clinical isolates of *Escherichia coli*. The aerobactin determinants from the latter source have been cloned and shown to occur in an operon preceded by a regulatory element [56]. Aerobactin, which consists of citrate substituted on the distal carboxyls with residues of *N*6-hydroxyacetyl lysine, is fabricated in sequence by oxidation of L-lysine, followed by acetylation and condensation, in a particular order, of two of these side chains with citrate. Four gene products are required for the biosynthesis. Work has centered on the gene encoding the monooxygenase since this enzyme catalyzes the first step in the pathway and is a logical target for chemotherapeutic intervention aimed at blocking aerobactin synthesis. The gene has been sequenced [34], and fusions with b-galactosidase were used as a means of solubilizing the enzyme [78]. Lysine-*N*6-hydroxylase, which carries loosely bound FAD, oxidizes the substrate at the expense of NADPH and molecular oxygen [45].

Siderophore production and extraction

Siderophore can be produced using iron restricted medium, however many researcher have produced bacterial siderophore by using succinate medium [50], the fermented succinate broth showing siderophore production shown.After complection of incubation the siderophore were extracted by [61], method and the crude siderophore crystals obtained by solvent extraction method shown.

Biocontrol agent

Many bacteria suppress the growth of deleterious microorganisms by production of siderophore, antibiotics, and cyanide [21]. Siderophore are themselves growth inhibitors of various phytopathogenic fungi such as *Phytophthora parasitica* [73], *Fusarium oxysporum veri dianthi* [33,9] and *Sclerotinia sclerotiorum* [48,37]. Were the first to demonstrate the importance 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 Pseudomonas* and their capacity to inhibit germination of chlamydospores of *F.oxysporum* [22]. Such as pyoverdine, were reduced in their capacity to suppress different plant pathogens [36,43].

Environmental applications

Iron is ubiquitous in almost all environments of this world. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pd and Ni. Most 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 environmental and may also used to treat radioactive waste prior to storage or to decontaminate soils and water [69,83].

Iron in the Environment

The aerobic atmosphere of the planet has caused the surface iron to become converted to oxyhydroxide polymers of very sparing solubility. The concentration of free ferric iron at neutral pH is dictated by the solubility product constant of ferric hydroxide. Depending on the value selected for this constant, the maximum amount of uncompleted ferric iron in solution at biological pH is probably not greater than 10^{-18} M [59]. These environmental restrictions and biological imperatives have required that microorganisms form specific molecules that can compete effectively with hydroxyl ion for the ferric state of iron, a nutrient which is abundant but essentially unavailable. Their biosynthesis is tightly controlled by levels of iron; for example, their production is repressed when iron is abundant in the environmental. Among the alternative means of assimilating iron are surface reductions to the more soluble ferrous species, lowering thepH, utilization of heme or extraction of protein-complexed metal. The role of these compounds is to scavenge iron from the environmental and to make the mineral, which is almost always essential, available to the microbial cell.

Iron in the Microbiology

Microorganisms growing under aerobic conditions need iron for a variety of functions including reduction of oxygen for synthesis of ATP, reduction of riptide precursors of DNA, for formation of heme, and for other essential purposes. A level of at least one micro molar iron is needed for optimum growth [65].Bacteria and fungi, in response to low iron availability in the environment, synthesize microbial iron chelates called siderophores. Siderophore are among the strongest soluble Fe^{3+} binding agents known [54]. Siderophore are low molecular weight Fe^{3-} compounds and most contain either hydroxamates or catechloes as the iron- sequestering groups. This being particularly important when evaluating the potential of a strain for bio control [46,71].Specific ligand that is produced by aerobic bacteria and fungi growing under low iron conditions.Siderophore production by the presence ofFe containing minerals, with increasingly strongersiderophore produced in response to increasingly insoluble iron sources. Generally have only micromolar quantities of iron, a concentration low enough to induce siderophore production

[39]. Some lactic acid bacteria are not stimulated to greater growth with iron, they have no home enzymes, and the crucial iron-containing ribotide reductase has been replaced with an enzyme using as the radical generator. Other microbes need iron but grow anaerobically on Fe(II). While nearly all fungi make siderophores, both budding and fission yeast appear to be exceptions. Siderophore appear to be confined to microbes and are not products of the metabolism of plants or animals, which have their own pathways for uptake of iron.

Research in this field began about five been related to virulence mechanisms in microorganisms synthesize at least one siderophore. Siderophore have been related to virulence mechanisms in microorganisms pathogenic to both animals and plants. In addition, they have clinical application and possibly important in agricultural[88].To increase the eventual commercialization of bacterial as biocontrol agents depend on part to the understanding of the mechanisms involved in the antagonist interaction between bacteria pathogen and host plants[79]. Bacteria have been employed efficiently as bio control agents present time there are some commercial products in the market [85]. Nevertheless, the application of purified siderophores, as bacteriostatic or fungistatic in combination with other antibacterial factor will certainly raise a great.

Isolation of Siderophore

Siderophores are isolation of the common products of aerobic and facultative anaerobic bacteria and of fungi.Elucidation of the molecular genetics of siderophore synthesis, and the regulation of this process by iron, has been facilitated by the fact that *E. coli* uses its own siderophores as well as those derived from other species, including fungi. Overproduction of the siderophore and its transport system at low iron is in this species well established to be the result of negative transcriptional repression, but the detailed mechanism may be positive in other organisms. Siderophores are transported across the double membrane envelope of *E. coli* via gating mechanism linking the inner and outer membranes.

Since siderophores differ substantially in structure, no uniform procedure is available for their isolation. A preliminary examination by paper electrophoresis should reveal the charge profile as a function of pH, following which appropriate exchange resins can be applied for retention and elution of the compound. Most are water-soluble, and it is thus usually expedient to drive the siderophore into an organic solvent, such as benzyl alcohol or phenol-chloroform, in order to eliminate salt. The siderophore may be isolated per se or as its iron chelate. The latter has the advantage of visual color, but the iron must be removed before any natural product can be characterized Vigorous hydrolysis in the presence of iron will destroy oxidizablemoieties, and direct NMR analysis is ruled out by the Para magnetism of the ferric ion[81]Both of these techniques are sensitive and capable of providing absolute answers. Less than half of the known siderophores will crystallize; otherwise X-ray diffraction is the method of choice since it affords the configuration of those molecules containing a chiral center [4].

Bacterial Siderophores

There are a few reports of finding in vivo expression of siderophores by bacterialzoo pathogens. For example, siderophores have been detected in sputum samples from the lungs of cystic fibrosis patients with infections due to *Pseudomonasaeruginosa* [31] and enterochelin has been found in peritonealwashings of guinea pigs infected with *Escherichia coli* [28].Immunoglobulins to siderophores have been detected in some instances.Such a host response is indicative of in vivo synthesis of iron chelators by somepathogens [66].When we look more deeply into the large group of marine *Vibrios*, we notice that a broad range of structurally different siderophores is produced [20]. Thus, catecholate siderophores have been detected in *Vibrio cholerae*, *Vibrio vulnificus* and *Vibrio fluvialis*. A mixed-type catecholate-thiazoline-hydroxamate siderophore, named anguibactin has been isolatedfrom *Vibrio anguillarun* and the citrate-based hydroxamate, aerobactinhas been described in certain marine vibrios. Also the occurrence of ferrioxamineG has been reported in vibrio species and we have recently identifiedthe structurally related dihydroxamate, bisucaberin, in the fish pathogen *Vibriosalmonicida* [87]. This broad range of structurally differentsiderophores in the family Vibrionaceae may reflect the existence of a largepool of siderophore biosynthetic genes and may also indicate that the different genera of the family are more heterogeneous than previously assumed; vibrioare widespread in marine water, but this does not necessarily mean that theyare really free-living bacteria. We may assume that most vibrios are somehow associated with particles in marine coastal water. Siderophores have also been isolated from several other marine bacteria, like *Alteromonas*, *Halomonas* and *Marinobacter*, indicating that siderophore production in the marine environment is widespread [47]. If we consider siderophore production within different microbial genera, we realize that catecholate siderophores predominate in certain Gram-negative genera, like the Enterobacteria and the genus *Vibrio*, but also in the nitrogen-fixing *Azotobacteria* and the plant-associated Agrobacteria. The reasons that these bacteria use catecholates may be manifold.

Siderophore produced in *Pseudomonas*

Bacteria belonging to the genus *Pseudomonas* are largely distributed in nature and can be isolated from most environments including soil, plant *rhizosphere* and *phylosphere*, or water. A few species are pathogens for animals, e.g., *Pseudomonasplecoglossicida* [60] or are, like the genus type-species *Pseudomonas aeruginosa*, opportunist human pathogens implicated in severe illnesses like cystic fibrosis. A greater number are plant pathogens, mainly found on the surfaces of plant leaves and stems such as *Pseudomonas syringae* and the related species *Pseudomonas amygdali*, *Pseudomonas avellanae*, *Pseudomonascannabina*, *Pseudomonas ficuserectae*, *Pseudomonas meliae*, *Pseudomonas savastanoi*,*Pseudomonas tremae* and *Pseudomonas viridiflava*[25]. Others, e.g., *Pseudomonas palleroniana* and *Pseudomonas salomonii* [24], or *Pseudomonas tolaasii* and *Pseudomonas costantinii*[55],have been associated with serious crop damages affecting rice, garlic or mushrooms, respectively. However, most of the *Pseudomonas* spp. remains to be considered as non-pathogenic saprophytic bacteria, harboring for many of them behaviors of biotechnological interests such as chemical bioremediation, crop protection or plant growth promotion. In soil, *Pseudomonads* represent one of the most important Gram-negative genera among culturable aerobic bacteria usually found. According to student lab courses done on soil samples over many years in our laboratory, 1–10% of soil isolates correspond to bacteria easily recognized as *fluorescentPseudomonas* thanks to the yellow-green, highly *fluorescent* halo existing around such colonies growing on the iron-poor Casamino acid (CAA)-agar medium. Some of these bacteria have been recognized as efficient plant growth

promoting rhizobacteria (PGPR), exercising a powerful biological control on soil-borne phytopathogens [32]. Iron competition was first thought to be the major mechanism responsible of the antagonistic effects of *Pseudomonads* towards pathogenic microorganisms [37]. However, antifungal antibiotic synthesis as well as plant elicitor production suggested that the *Pseudomonad* PGPR effects were not restricted to siderophore-mediated iron competition [32].

Diversity within the genus *Pseudomonas*, first mainly established on the basis of a restricted panel of phenotypic traits[62], has been largely developed since the last decade with more than 30 new *fluorescent Pseudomonas* species described thanks to the application of polyphasic taxonomy [82]. Today, the definition of a novel *Pseudomonas* species or, more frequently, the characterization of a *Pseudomonas* isolate at thespecies level, requires, in order to be successful, the use of numerous phenotypic and genomic methods, which means much investment in time, labor, material and money. Such practical considerations should explain why some of the earliest defined and most important species like *Pseudomonas fluorescens* or *Pseudomonasputida* are still commonly in use, although well recognized by taxonomists as groups formed by a complex of species [91,6]. Siderotyping methods, in use in our laboratory for many years, proved to be rapid, accurate and inexpensive tools for pseudomonad characterization and identification [52]. Interestingly, three of these sub-groups correlated with newly described *Pseudomonas* species, i.e., *Pseudomonas jessenii*, *Pseudomonas mandelii* and *Pseudomonas rhodesiae*, respectively, while the most important sub-group by numberof isolates was considered as corresponding to the *P. fluorescens* sensu strictospecies, since containing the type strain *P. fluorescens* ATCC 13525 among itsrepresentatives. Other sub-groups could be considered as potential new specieswhich remain to be defined. Such an example well illustrates the efficiency andusefulness of siderotyping in *Pseudomonas* biodiversity studies as well as in taxonomystudies.

Fungal Siderophores

Two major responses to iron stress in fungi are a high-affinity ferric iron reductase and siderophore synthesis. Uptake of siderophores is a diverse process, which varies among the different classes of compounds. Three common classes – phenolates, hydroxamates, and polycarboxylates – are observed. Some phytopathogenic fungi produce unique compounds that function as phytotoxins but also chelate iron.*Curvularia lunata* is a fascinating fungus exploited by authors for transformationof several steroids and an antibiotic [76,11,10],has isolated four siderophores namely neocoprogenI, neocoprogen II, coprogen and ferricrocin from defer rated sucrosemedium. When the media was inoculated with spore suspension and incubatedon a rotary shaker at 220 rpm, high siderophore production was observed in120 h. During logarithmic growth of the organism, there was gradual and slowincrease in concentration of siderophore; however, after initiation of the stationaryphase there was an abrupt increase in siderophore concentration. Addition ofexogenous iron inhibited production of siderophore at concentration of 50µM.Siderophore production was optimum at pH 6.

Characteristicsof Siderophore

Siderophores form high-spin, kinetically labile chelates with ferric ion which are characterized by exceptional thermodynamic stability [70,64]. The formation constant for typical molecules containing three bidentate ligands is 10^{30}, or greater. The affinity for gallium is also high, but the attraction for aluminum and for all divalent ions is substantially less. Thus, the siderophore ligand can be said to be "virtually specific" for Fe(III) among the naturally occurring metal ions of abundance. Synthetic man-made elements in the actinide series are also firmly bound.

However, soil-borne bacterial and fungal pathogen diseases cause severe losses annually because of succession planting. So effective plant growth-promoting rhizobacteria (PGPR) including siderophore-producing bacteria were used to prevent infectious diseases of plant roots. Siderophores are low molecular weight (generally<10, 000Da), iron-coordinating, organic compounds produced by most aerobic and facultative anaerobic microorganisms to combat low-iron stress. Data showed that the excretion of siderophores by rhizosphere bacteria may stimulate plant growth by improving Fe nutrition of the plants or by inhibiting the establishment of plant pathogens or other harmful microorganisms. Production of siderophore and antifungal activity were simultaneously exhibited by free-living rhizospheric isolates of *Azotobacter* (16.22%), fluorescent *Pseudomonas* (11.11%) and *Bacillus* (10%). The siderophore-producing *Pseudomonas*SF4c can increase the shoot and dry biomass of wheat by 23% and 45% under greenhouse conditions, respectively. Iron is vital element require by all living organisms for many cellular processes such as electron transport chain and as a cofactor for many enzymes. Microorganisms growing under aerobic condition including reduction of formation of home andforother essential purposes. The aerobic atmosphere of the planet has caused the surface iron to oxidize to insoluble oxyhydroxide polymer and reduced the level of free iron. Therefore microorganisms adopted a way for iron acquisition by producing iron chelating molecule i.e Siderophore. Siderophore are low molecular weight (<10 D) iron chelating compounds synthesized by many bacteria *Pseudomonas, Azotobacter , Bacillus, Enterobacter, serratia,Azospirillum* and *Rhizobium* [27],in large quantity under iron limited conditions.A great variation is seen in siderophore structure from one three main kinds of siderophore known as hydroxamate, catecholate and carboxylate.But since some of the ensuing discussion involves methods employed by investigators, a brief summary is given here. The siderophores are produced in large excess by fungi in ironstarved cultures and turn red upon capture of Fe(III). Thus, a limited-iron medium for the fungus of our choice needs to be designed [13]; the fungus is grown in the limited-iron medium, periodically iron salts are added to a centrifuged sample, and when a sample turns red, the culture medium is harvested for further processing.

There are a few reports of finding in vivo expression of siderophores by bacterial zoopathogens. For example, siderophores have been detected in sputum samples from the lungs of cystic fibrosis patients with infections due to *Pseudomonasaeruginosa* [31], and enterochelin has been found in peritoneal washings of guinea pigs infected with *Escherichia coli* [28]. Immunoglobulins to siderophores have been detected in some instances. Such a host response is indicative of in vivo synthesis of iron cheater's by some pathogens [67]. When we look more deeply into the large group of marine *Vibrios*, we notice that a broad range of structurally different siderophores is produced [19]. Thus, catecholate siderophores have been detected in *Vibrio cholerae*, *Vibrio vulnificus* and *Vibrio fluvialis*. A mixed-type catecholate- thiazoline-hydroxamate Siderophore, named anguibactin, has been isolated from *Vibrio anguillarun* and the

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If we consider siderophore production within different microbial genera, we realize that catecholate siderophores predominate in certain Gram-negative genera, like the *Enterobacteria* and the genus *Vibrio,* but also in the nitrogen-fixing *Azotobacteria* and the plant-associated Agrobacteria. The reasons that these bacteria use catecholates may be manifold. However, lipophilicity, complex stability, high environmental pH and a weak nitrogen metabolism might favour catecholates. The Gram-positive *streptomycetes* produce hydroxamate-type ferrioxaminesand the ascomycetous and basidiomycetous fungi synthesize ester and peptide-containing hydroxamate siderophores that are acid-stable and well suited for environmental iron solubilization. Both the *Streptomycetes* and fungi show a versatile nitrogen metabolism with active N-oxygenases.

Siderophores are common products of aerobic and facultative anaerobic bacteria and of fungi. Elucidation of the molecular genetics of siderophore synthesis, and the regulation of this process by iron, has been facilitated by the fact that *E. coli* uses its own siderophores as well as those derived from other species, including fungi. Overproduction of the siderophore and itstransport system at low iron is in this species well established to be the result of negative transcriptional repression, but the detailed mechanism may be positive in other organisms. Siderophores are transported across the double membrane envelope of *E. coli* via a gating mechanism linking the inner and outer membranes.

Medicinal application

The obligate nutritional requirement for iron by multiple drug resistant pathogens can be exploited to control its infection. The three fundamental perceptions of iron dependent pathogen control include the Trojan horse concept to facilitate the cellular uptake of antibiotics, artificial iron starvation by using siderophores or antagonists that cannot be utilized as an iron source by the pathogen and inhibition of iron metabolism pathways[3].

Iron overload diseases β-thalassemia

In the treatment of β-thalassemia and certain other anemias, periodic whole blood transfusions are required [35]. Since there is no execration 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[63]. Desferrioxamine B has also found therapeutic application for various pathological conditions due to aluminum overload [1]. 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 component (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 microorganisms 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 [41,90].

Iron chelators and cancer

Siderophore potential used as iron chelators in the treatment of cancers e.g Dextrazoxane, O-trensox,desferriexochelins, desferrithiocin, tachpyridine, have been found in cancer therapy [53]. Also siderophore used for the clearance of non-trans ferric bound iron in serum which occurs in cancer therapy as a result of some chemotherapies [12].

Anti-Malarial

Some siderophore have been found to be useful in the treatment of malaria caused by *plasmodium falciparum.* Siderophore produced by *Klebsiella pneumonia* act as antimalarial agent [30]. Desferrioxamine B produced *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 cause intracellular iron depletion. The same siderophore was shown to inhibit growth of *Trypanosoma brucei*, another protozoic parasite causing sleeping sickness in human bloodstream [8].

Regulation

It has been known for many years that all components of siderophore systems are depressed at low levels of iron. The first report on the molecular genetics of the process came with work on *Salmonella typhimurium*. Chemical mutagenesis identified a gene, designated for(ferric uptake regulation), which controlled expression of the siderophore, again enterobactin, and a brace of large outer membrane proteins, one of which is the equivalent of FepA of *E. coli*[23]. In the latter organism, the gene was cloned and sequenced, and the product was isolated and shown to act as a classical negative repressor of transcription [2]. Although any first rowdivalent transition element will "organize" Fur to bind the operator, Fe(II) is thought to be the natural activator because of the relative abundance of iron. The "iron box" or "fur box"consensus sequence in the operator is GATAATGATAATCATTATC, an array which occurs with some variation in the regulatory DNA of iron-affected systems in many microbial species.Polymerization of Fur around the operator has been suggested as the mode of binding [17], and this is supported by observations with the electron microscope [38]. On the other hand,both Fur and ArcA, the latter the repressor for the soda gene coding for manganese-superoxide dismutase, bind at the same site. Footprinting experiments demonstrated polymerized binding in the 210 to 235 region of the promoter but suggested interaction with one face of the

double helix [77]. Theinteraction of metallo-Fur with DNA was reinvestigated, and it was concluded that the repressor, which lacks the classic helixturn- helix motif, contacts one face of the DNA across almostthree successive major grooves [15]Earlier it was establishedthat the N-terminal region of Fur recognizes DNA while otherdomains of the repressor are involved in separate functionssuch as binding metal or polymerization[14].A still baffling aspect is the fact that a number of genesseemingly unrelated to iron acquisition, in addition to that forsuperoxide dismutase, are also part of the Fur regulation. A Furtitration assay has been proposed as a means of identifying allgenes regulated by the repressor [75].In contrast to the straightforward regulatory mechanism ofthe aerobactin operon by ferrous-Fur, regulation of the *fur* geneitself seems considerably more baroque. As well as an iron box,sites for binding of CAP have been identified [18].The negative regulation scheme with Fe(II) as co-repressorfor a small, Fur-like protein appears valid in many other bacterialspecies such as in the iron-regulated formation of toxinby *Corynebacterium diphtheria* [7]. Some variation in thestructure of the repressor and the operator can be anticipated.However, in *Pseudomonads*, a positive mechanism may underliethe observed overproduction of the fluorescent siderophoresvariously known as *pseudobactins* and *pyoverdines* [68].The *fur* mutants of *E. coli* grow poorly, possibly becauseof oxidative stress [80]. The mutation appears to be lethal in*Neisseria* spp[5].

Conclusion

UnderConcentration of Fe (III) is the most important factor in the production ofsiderophores.We discussed in a literature review the important roles and applications of siderophores in different environmental habitats. Higher concentration of siderophore is produced under lowerconcentration of Fe(III). During the production of siderophore the pH changes but at the end of the culture generally the pH of the medium increases to alkaline values.The production of siderophores occurs under conditions of iron-limitation.Such conditions are likely to prevail in the *rhizosphere*[89], and siderophore mediated competition for iron is one of the mechanismsof bacterial antagonism against soil-borne pathogens[43].

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