Chapter 7
Contrasting Role of Fungal Siderophore in Metal Ion Complex Formation

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7.1   Introduction

Plants and microbes have vast significance in our day-to-day life. Iron is considered to be an abundant element in the soil (earth's crust) and essential for all life processes such as respiration, DNA synthesis, tricarboxylic acid cycle and production of various small molecules like amino acids, lipids, and sterols. Being an essential element in earth's crust, the bioavailability of iron is limited in the habitat (soil and sea) owing to its low solubility. This property of iron results in its poor uptake by plants, which eventually makes iron an essential nutrient for plant growth. In aqueous and oxygenated conditions, the iron is found in its supreme state, which accumulates in the form of minerals such as iron oxides and iron hydroxides and is not ready to be utilized as such by organisms. In order to overcome this restricted process, the microbial flora of soil such as *Pseudomonas* spp., *Enterobacter* genus, *Bacillus* spp. produces special iron-binding tiny carriers, called 'siderophore', that help to scavenge iron from these mineral phases (oxides and hydroxides) by forming soluble iron (Fe^{3+}) complexes which are readily taken up by the environment through active transport mechanism (Philpott 2006).

Siderophore came from Greek words sidero that means 'iron' and phore that means 'carriers', and in combination, it is termed as 'iron carrier'. Siderophores are small, low-molecular-weight (<10 kDa) iron-chelating compounds, secreted by plants and microorganisms (bacteria and fungi) to maintain their iron requirement. These are also produced by rhizospheric bacteria in iron-limiting conditions in order to increase the plant growth by scavenging iron from the environment and make it available to the cell near the plant roots (Sah and Singh 2015; Li et al. 2016).

7.2   Siderophore-Mediated Iron Transport

Iron transport in siderophore is an energy-dependent mechanism. Type and stereoselectivity of siderophore are specific factors in recognition and transport of iron-siderophore complexes in microbes. The complexation also depends on metal ion coordination geometry as well as N-acyl residues present at the periphery of central metal ion. For instance, the coordination of metal centre and configuration of ligand affect the stability of complex. In case of *Rhodotorula pilmanae*, configuration of macrocyclic rings of siderophore is favoured, whereas in contrast to this, in *Penicillium parvum*, *Neurospora crassa* and *Aspergillus quandricinctus*, L-*cis*-ferrichrome is found to be a stable configuration. Further, the geometrical stability of complex also depends on the types and number of N-acyl residues surrounding the iron coordination centre (Huschka et al. 1986).

In spite of having specific transport mechanism of siderophore, many microbes may utilize multiple transport system as well as more than one type of siderophore at a time for efficient transport of metal ion. For example, microorganism like *Agaricus bisporus* has variable transport systems for fusarinines and ferrichromes,

whereas *Neurospora crassa* represents different recognition sites for coprogen- and ferrichrome-type siderophore system (Howard 1999).

In fungal species, majority of literature suggested that *Saccharomyces cerevisiae* has two high-affinity iron transport mechanism. In first reductive mechanism, ferric iron (Fe^{3+}) is identified to be reduced by a number of inducible membrane-bound reductase, namely, Fre1p–4p, usually present at cell surface. Across plasma membrane, the reduced iron (Fe^{2+}) is then transported by involvement of permease-oxidase, namely, Ftr1p and Fet3p. This mechanism is utilized by variety of Fe^{3+}-siderophore complexes such as ferrichrome, triacetyl fusarinine C and rhodotorulic acid for their transportation after being reduced by cell-surface-bound reductase. On the other hand, the second mechanism involves the uptake of iron (Fe^{3+})-siderophore complex as an intact form into the cell. All the proteins that are involved in the transport of so far identified complexes by this type of mechanism belong to major facilitator superfamily, namely, Sit1p (also known as Arn3p which helps in transporting ferrioxamine B, ferrichrome and ferrichrome A), Arn1p (helps in transporting ferrirubin, ferrirhodin and ferrichrome A), Taf1 (also known as Arn2p, which helps in transporting triacetyl fusarinine C) and Enb1p (helps in transporting enterobactin). The uptake specificity may vary among receptors as well as among strains (Renshaw et al. 2002). For instance, Arn1p specifically transports ferrichrome-type siderophores around the iron centre which have branched-chain ornithine-N5-acyl residues but does not support the short-chain acetyl hydroxamic residues present in siderophore such as ferrirubin and ferrichrome. Likewise, Arn2p is found to specifically transport triacetyl fusarinine C, whereas Sit1p has been found to be less specific. Arn1p and Sit1p transport the complex via cell surface and are then rapidly internalized as both are localized in intracellular vesicle layers (Seneviratne and Vithanage 2015). Table 7.1 represents the list of siderophore transport supported by different receptors.

Table 7.1 Various receptors involved in siderophore transport in fungi (Bairwa et al. 2017; Raymond 1994)

Fungal species	Protein/receptors	Siderophore transported
S. cerevisiae	Arn1	Ferrichrome and ferrichrome A transport
	Arn2/Taf1	Triacetyl fusarinine C transport
	Arn3/Sit1	Ferrichrome and ferrichrome A transport
	Arn4/Enb1	Enterobactin transport
Candida albicans	Arn1/Sit1	Ferrichrome-type xenosiderophore transport
C. glabrata	Sit1	Ferrichrome transport
Cryptococcus neoformans	Sit1	Ferrioxamine transport
A. fumigatus	Sit1	Ferrichrome and ferrioxamine B transport
	Sit2	Ferrichrome transport
	MirB	Triacetyl fusarinine C transport
Histoplasma capsulatum	Mfs1, Abc1	Putative siderophore transporter
Rhizopus oryzae	Fob1, Fob2	Ferrioxamine binding at cell surface

7.3   Fungal Iron Regulation

In fungi, regulation of iron uptake is necessary to maintain iron homeostatic processes. For this, four different mechanisms of iron uptake have been suggested in fungi at molecular level by different mechanistic-based studies (Haas 2014). These four mechanisms involve (a) ferric iron (Fe^{3+}) uptake through siderophores, (b) reductive iron assimilation, (c) heme uptake and d) direct iron uptake. These four mechanisms are explained as follows.

7.3.1   Iron (Fe³⁺) Uptake Through Siderophore

Each and every fungal species exhibit siderophore-iron transporter (SIT)-mediated extracellular iron uptake mechanism. SIT constitute majorly facilitator protein family, which acts as a proton-coupled symporters potentiated by plasma membrane. In addition, high solubility and high energy factors render iron-chelated siderophore to combat during microbial growth. On the other hand, triacetyl fusarinine (TAFC) and fusarinine C (FsC) facilitate intercellular release of iron by partial hydrolysis by esterase (Estb) enzyme (Howard 1999).

7.3.2   Reductive Iron Assimilation (RIA)

In fungi, iron acquisition with high affinity is usually achieved either by secreted siderophores (iron chelators) or by reductive iron assimilation (RIA) mechanisms (Fatima et al. 2017). In order to start iron uptake, iron is first reduced from ferric (Fe^{3+}) to more soluble ferrous (Fe^{2+}) form by localized ferrireductases present in the plasma membrane of fungal species. Soon after this, the ferrous iron is re-ionized which is further imported by protein complex consisting of iron permease (FtrA) and ferrioxidase (Fetc) genes. The protein such as permease also transports metals other than iron such as copper and zinc.

7.3.3   Heme Uptake

In contrast to bacteria, binding and uptake of iron, in fungi, are done only with heme component. For instance, *Candida albicans* heme uptake mechanism involves the glycosylphosphatidylinositol (GPI)-anchored cell surface mannoprotein, namely, Rbt5P, but the details regarding its transport mechanism are still unknown. In order to utilize heme-iron complex, the uptake requires intercellular degradation of heme with heme oxygenase (Hmx1p, localized in endoplasmic reticulum).
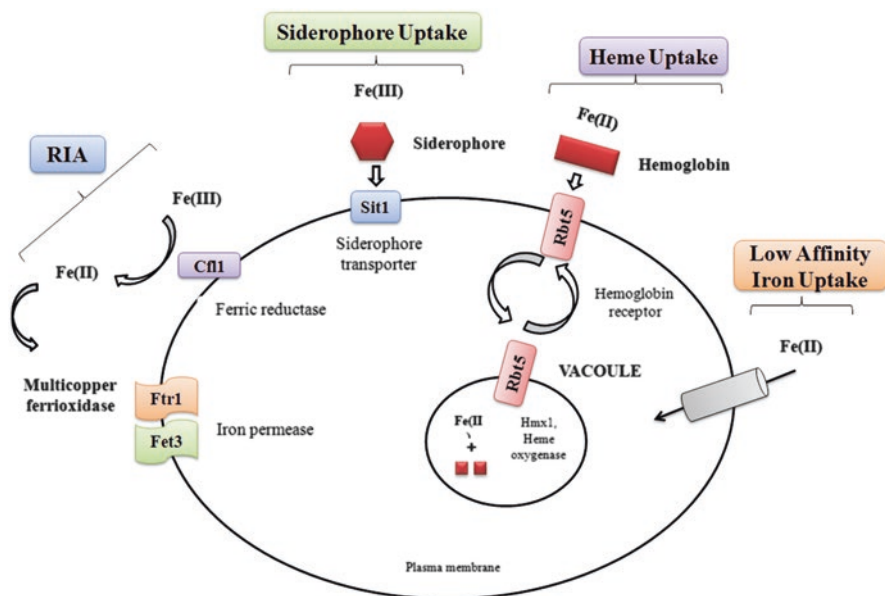
Fig. 7.1 Different approaches in fungal iron acquisition

7.3.4 Low-Affinity Iron Uptake

The iron in the form of ferrous (Fe^{2+}) is taken up by permease Fet4p. The system is non-specific for Fe^{2+} form of iron as it also transports other metals such as zinc and copper. In *Saccharomyces cerevisiae*, the iron supply is associated with mobilization of iron from vacuole which is facilitated by fluid-phase endocytosis and Smf1p protein belonging to natural resistance-associated macrophage protein (NRAMP) family (Kosman 2003).

All the four iron regulation mechanisms are pictorially represented in Fig. 7.1.

7.4 Types of Siderophore

Chemically based on the interaction sites, siderophore has been categorized into two main groups, namely, first 'enterobactin', the strongest iron chelator which shows interaction between iron and catecholate hydroxy groups, and second 'hydroxamate' that acquires N-hydroxylated amide bonds (e.g. ferrichromes in fungi). Atoms like nitrogen (N), oxygen (O) and sulphur (S) can also participate in the coordination of iron in carboxylate groups (Drechsel et al. 1995; Butler and Theisen 2010). Mixed type of siderophores involves those donating groups that do not belong to hydroxamates or aromatic hydroxy group category. The description for various types of siderophores (shown in Fig. 7.2) is summarized as follows.
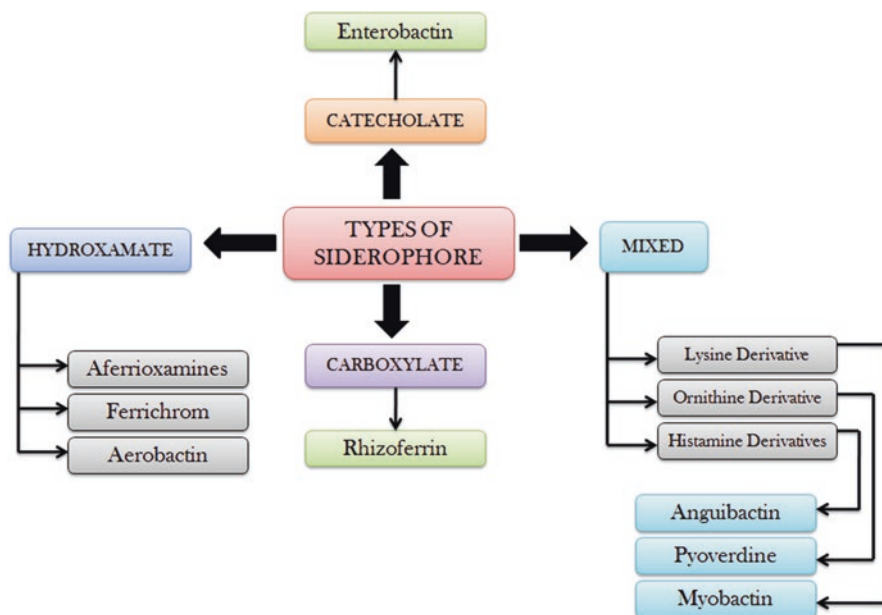
Fig. 7.2 Schematic representation of different types of siderophores

7.4.1 Hydroxamate Siderophores

Chemically, these are specifically tri-hydroxamate type of siderophores reportedly found in fungi and majorly produced by fungal species that belongs to Zygomycotina (Mucorales), Ascomycotina (*Aspergillus* spp., *N. crassa*) and Deuteromycotina (*Fusarium dimerum*) subdivision of fungi. Hydroxamate siderophores have strong correlation among their hydroxamate groups and bound ligands as they known to form hexadentate, tetradentate and bidentate complexes. In hydroxamate class mainly, ferrichromes are of main concern with respect to ecological importance due to their potential to chelate iron from soil and supply to plant species, whereas other naturally occurring siderophores are not as effective because of their affinity towards other metal ions too. From the literature reports, ferrichrome derivative such as ferrioxamines also exhibits antibiotic activity (named as ferrimycines). In addition, rhodotorulic acid, dimerium acid, alkaligens and putrebactin also belong to hydroxamate-type siderophore family (Garnerin et al. 2017).

7.4.2 Carboxylate Siderophores

A new class of siderophore was identified by Winkelmann from fungi *Rhizopus microspores* belonging to zygomycetes, which contain 'hydroxyl' and 'carboxyl' moieties as donor groups to iron (Fe^{3+}) which are solely responsible for metal bind-

ing. This type of siderophore is termed as 'carboxylate siderophore' well known as 'rhizoferrin' which is isolated from *Rhizopus* spp. using ion-exchange column chromatography. Structurally, rhizoferrin and its analogues contain 1,4-diaminobutane symmetrically acylated to the terminal carboxylate of citric acid through amide bonds (Drechsel et al. 1995).

7.4.3 Catecholate Siderophore

This class of siderophores is known to have phenolate or 2,3-dihydroxybenzoate (DHB) as a binding moieties. Catechol (also called as pyrocatechol), naturally occurring organic, colourless compound, is the ortho-isomer of the three isomeric benzenediols and found in trace amounts. *Azotobacter vinelandii*, in iron-deficient medium, forms various types of catecholate-based siderophores such as monocatecholate aminochelin, dicatecholate azotochelin and tri-catecholate protochelin (Baakza et al. 2004). Basically, all these types of naturally occurring siderophores contain negatively charged oxygen donors as hard Lewis base which binds with Fe^{3+} which act as hard Lewis acid as per its chemical nature.

7.4.4 Mixed Ligand Siderophores

This class of siderophores, also called heterobactins, contains combined donor groups of hydroxamate and catecholate together. Siderophore of this type includes mixed ligand of lysine, ornithine and histamine derivatives. For instance, mycobactins (*Mycobacterium* spp.) contain hydroxamate and phenolate donor groups as chelating ligands (Mohammad et al. 2011).

7.5 Mechanism of Binding of Iron in Cell

In fungi, siderophore-mediated iron (Fe^{3+}) uptake can be regulated by four mechanisms, namely, shuttle, hydrolytic, taxicab and reductive. Three of them, i.e. shuttle, taxicab and hydrolytic mechanisms, depend upon the specific recognition of several siderophore. (a) In shuttle mechanism, the iron (Fe^{3+})-siderophore complex initially enters the cell membrane, and soon after that, it releases the metal from ligand (e.g. ferrichrome in fungal species, viz. *Ustilago sphaerogena* and *Ustilago maydis*) which ultimately leads to excretion of free siderophore (Sah and Singh 2015). This mechanism is depicted in Fig. 7.3.

The hydrolytic mechanism involves the transportation of iron (Fe^{3+})-siderophore complex in its intact form into the cell (e.g. uptake of ferric triacetyl fusarinine C in *mycelia sterilia*). Simultaneously, both reductive and degradative steps occur inside

the cell that lead to the reduction of iron from ferric (Fe^{3+}) to ferrous (Fe^{2+}) form and also the cleavage of ester bonds in siderophore (triacetyl fusarinine C) in the presence of specific esterase resulting in monomeric fusarinines which are further excreted as shown in Fig. 7.3. This mechanism is helpful for removing toxic metals such as aluminium (Al^{3+}), gallium (Ga^{3+}) and chromium (Cr^{3+}) from the cell as these metal-siderophore chelates have similarity to the prototype iron (Fe^{3+}) complex and their complexes are taken up inside the cell where these metals remain bounded to the monomeric fusarinine, so further reduction of these metals is restricted resulting in their excretion (Sanz-Ferramola et al. 2013), whereas, (c) in taxicab mechanism, iron in ferric form is transferred from extracellular siderophore to intracellular ligands (e.g. ferric rhodotorulate in *Rhodotorula pilimanae*) across the cell membrane as the extracellular siderophore does not enter the cell membrane (Gerwien et al. 2018). However, (d) in reductive mechanism, the reduction of iron (Fe^{3+})-siderophore complex occurs at the membranes instead of transporting that complex inside the cell, and the reduced ferrous form is taken up by the cell membrane. This type of mechanism is used for transporting ferrichrome siderophores in some fungal species such as *Ustilago maydis* (Trivedi et al. 2016).
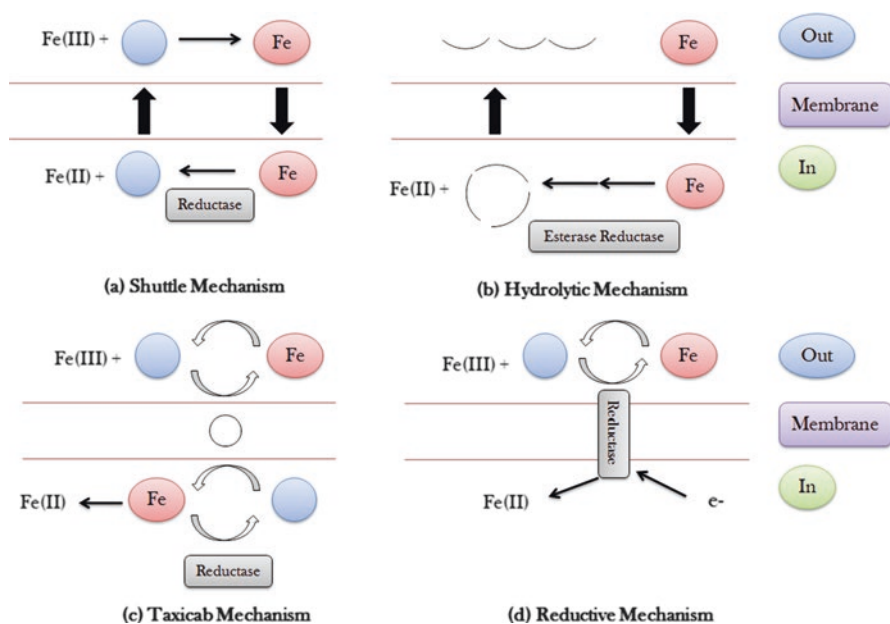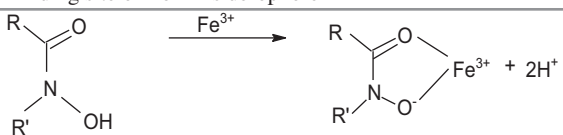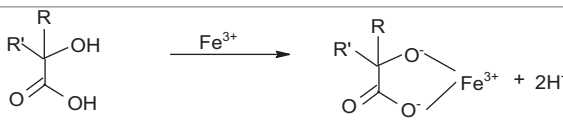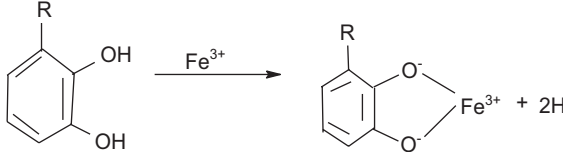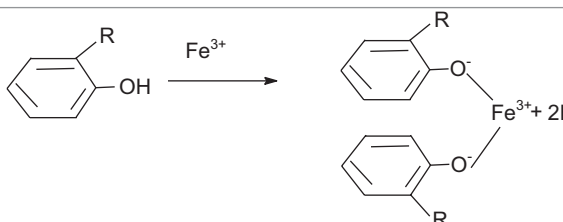

Fig. 7.3 Depiction of various types of iron uptake mechanism via siderophore across cytoplasmic membrane in fungi

7.6   Binding Sites in Siderophores

The stability of iron-siderophore complex strongly depends on the nature of binding sites present in varied siderophore structure with different functional motifs. All the different types of siderophores reported till date form extremely stable, highly specific complex containing high-spin ferric iron. The different moieties that explain the anchoring in most siderophore-iron complex belong to hydroxamates, catecholate, carboxylate and mixed-type categories as shown in Table 7.2. The first three types show some similarities in respect to their iron-binding affinity. These three classes hire two sites of the iron centre that form stable five-membered ring structure complexing metal iron to form siderophore-iron complex. Oxygen shows high affinity towards iron (Fe^{3+}), and also classified under hard donor ligand, further, a lone pair of oxygen atom always coordinates with iron cation in order to increase the strength of coordination complex. Using this donor-acceptor interaction, the selectivity of siderophore ligand for iron improves many folds, and this helps in transportation of iron across the cell membrane. Mixed type of siderophore consists of different binding sites. For example, rhizoferrin, a fungal siderophore, contains two molecules of citric acid usually linked with an additional chain. Further, it requires two hydroxycarboxylic acid and two carboxylic acid groups for iron coor-

Table 7.2 Binding site of iron in different types of siderophore (Wittenwiler 2007)

Sl. no	Class of siderophore	Binding site of iron in siderophore
1.	Hydroxamate	
2.	Carboxylate	
3.	Catecholate	
4.	Mixed ligand (phenolate type)	

dination (Neilands 1995). Table 7.2 illustrates the structural representation of iron-siderophore complex among different classes of siderophore.

The physico-chemical parameter, i.e. pKa values of binding groups, has a great influence on the siderophore's complex stability as the oxygen atom in these complexes is interacting in a non-protonated state. From the available literature, hydroxamic acid has been shown to have pKa values in the range of 8–10; however, acetohydroxamic acid (main compound for monohydroxamate ligand) has shown to have 9.29 pKa value. The pKa values of carboxylates lie in the range of 3.5–5 which contributes to efficient iron imbibitions by carboxylate siderophores under low-pH conditions. The microbes living in acidic medium (such as fungi) use carboxylate siderophores for iron immobilization. In spite of this fact, the carboxylate siderophores couldn't compete with stronger siderophores such as hydroxamates and catecholates as they are fully protonated at physiological pH. In catecholate, a model monomer has been synthesized in such a way that once nitro group is added on para position, then the pKa values of resulting alcohol groups were found to be 6.69 and 10.83. On the other hand, after substituting it with hydrogen atom, the resulting pKa values were 9.26 and 13.3. The pKa values of binding groups have an impact on the affectivity of siderophores as the oxygen atoms only bind with the groups in non-protonated state. These values are not easily accessible and effected by side chain modifications (Neilands 1995). The pKa values for hydroxamate, catecholate and carboxamate groups exhibit the partly deprotonated states in the presence of iron at neutral pH. Proton-independent pKa values do not demonstrate the actual iron-binding efficiency of siderophores at physiological pH due to incomplete deprotonation. For better analysis, pH analogous to pFe values is considered to be a better approach for comparing the true relative abilities of iron binding with different siderophores by giving negative decadic logarithm of free iron concentration. As per standards, the total ferric concentration and total ligand concentration are considered to be 10^{-6} M and 10^{-5}, respectively. The chelation efficiency is strongly influenced by pH of medium; thus, pFe value is a pH-dependent value. For instance, at serum pH (i.e. 7.4), in the presence of enterobactin and aerobactin, the concentration of free iron is observed to have different pFe values which are 35.5 and 23.4, respectively (Wilson et al. 2016; Miethke and Marahiel 2007).

Phytosiderophores are hexadentate ligands that coordinate with Fe^{3+} from all six coordination sites which explore a variety of combinations at the three binding sites to form a potential iron-siderophore complex. In the contrary, many siderophores use only one type of binding site to form the stable complexes with iron such as tri-hydroxamate siderophore (ferrioxamine) and tri-catecholate siderophores (enterobactin) (Renshaw et al. 2002).

7.7 Metal Ion Complex Formation with Siderophore

Several fungal species have been investigated with complex regulatory arrangements during intake of secondary metabolites, namely, mycotoxins (produced by mycotoxigenic fungi) and its detoxification process of converting these metabolites

from more toxic to less toxic form. The metals such as iron, zinc, copper, chromium, gallium and manganese interact with the siderophore produced by different fungi to make stable siderophore-metal complexes resulting in homeostasis regulation. These metal ions may interact with variety of siderophore. Table 7.3 highlights some of the examples of metal-ion-siderophore complexes along with their functions.

Table 7.3 Different metal ion complexes with siderophores

Sl. no.	Metal ion	Fungal source	Siderophore and derivatives	Functions	References
1.	Iron (Fe)	*Saccharomyces cerevisiae*	Catecholate, hydroxamate, ferrioxamine, ferricrocin	Facilitates two types of high-affinity iron transport systems, namely, reductive and non-reductive transport	Senthilnithy (2008)
2.	Iron (Fe)	*Paracoccidioides yeast*	Hydroxamates	Target for antifungal chemotherapy	Lesuisse et al. (1998)
3.	Iron (Fe)	*Trichoderma* species	Hydroxamates, carboxylates	Crop enrichment, improvement of medical interest and management of fungal disease on crops	Baila et al. (2014)
4.	Iron (Fe)	*Aspergillus* species, *Penicillium oxalicum*, *Aureobasidium pullulans*, *Phanerochaete chrysosporium*	Hydroxamates	Iron mobility and transport through soil	Ghosh et al. (2017)
5.	Iron (Fe-III)	*Rhizopus microspores*	Carboxylates (rhizoferrin)	Relieve the iron-restricted growth by iron transport into the cells of the indicator strains	Drechsel et al. (1995)
6.	Iron (^{55}Fe) with 13C-desketoneoenactin	*Candida* species	Ferrichrome, hydroxamates	Drug targeting and promotes growth in an iron-restricted medium	Baakza et al. (2005)
7.	Cobalt (Co-II/III)	*Penicillium brevicompactum*, *Aspergillus fumigates*	Hydroxamates (desferricrocin, desferrioxamine, desferricoprogen, tri-acetyl fusarinine)	Antifungal effect	Bernier et al. (2005)
8.	Gallium-68 (^{68}Ga)	*Aspergillus fumigates*	Hydroxamates	Infection imaging	Farkas et al. (2018)

Table 7.3 (continued)

Sl. no.	Metal ion	Fungal source	Siderophore and derivatives	Functions	References
9.	Gallium-68 (^{68}Ga), zirconium-89 (^{89}Zr)	*Aspergillus fumigates*	Ferrichrome, ferrichrome A, tri-acetyl fusarinine C, desferrioxamine, desferrioxamine E, coprogen, fusarinine C, ferricrocin	Infection imaging	Petrik et al. (2012a, b), Haas et al. (2015) and Petrik et al. (2010, 2014, 2015)
10.	Gallium-67 (^{67}Ga)	*Ustilago sphaerogena*, *Aspergillus fumigates*	Ferrichrome, ferrichrome A, rhodotorulic acid, tri-acetyl fusarinine C, malonichrome, desferrioxamine	Significant iron transport in microbes	Petrik et al. (2016), Emery and Hoffer (1980), Conti and Eriksson (2016) and Velikyan (2014)
11.	Chromium (Cr)	*Ustilago sphaerogena*	Desferrioxamine	Analogous to iron-siderophores that competitively inhibit utilization of iron-siderophore	Stintzi et al. (2000)
12.	Gallium-67 (^{67}Ga), indium-111 (^{111}In)	*Escherichia coli*, *Salmonella typhimurium*	Enterobactin (tricatecholamide analogue)	Ligand for radiopharmaceuticals	Crowley et al. (1988) and Petrik et al. (2017)
13.	Cobalt (Co)	*Fusarium solani*	Ferricrocin	Synthesized intercellular siderophore for iron storage, and highlighted the importance of iron tolerance against cobalt	Moerlein et al. (1981)
14.	Zinc (Zn)	*Candida albicans*, *Aspergillus fumigates*	Zincophores	Significant for identification of novel virulence factors in microbes	Rasha (2017)
15.	Zinc (Zn) and copper (Cu)	*Fusarium* species	Ferricrocin	Imposed toxic effect to the fungi present in soil	Wilson et al. (2012)
16.	Zinc (Zn), copper (Cu) and iron (Fe)	*Rhizophagus irregularis*	Enterobactin, coprogen	Significant for plant colonization	Islam and Datta (2015)
17.	Copper (Cu II), manganese (Mn-II), zinc (Zn-II), magnesium (Mg-II)	Mucorales, *aspergilli*, *penicillia*, *Neurospora crassa*, *Fusarium dimerum*	Hydroxamates, carboxylates	Improved binding affinity of siderophore	Tamayo et al. (2014)

7.8 Biological Functions of Siderophore

Siderophore affects the plant and microbes in several significant ways. These are discussed in detail as follows.

7.8.1 An Iron-Scavenging Compound

Siderophores have the ability to support the growth factor in all fungi and auxotrophic organism despite the fact that they are producing or non-producing species. Several auxotrophic organisms found to be in underdeveloped stage in the absence of these compounds require siderophores for their growth. For instance, *Pilobolus kleinii* utilizes coprogen as an essential growth factor (Dave and Dube 2000). The siderophore-mediated transport system facilitates and enhances the efficient competency among microbes for the consumption of available iron to make survival of one type of microbe over the other. This is advantageous in case of non-pathogenic species producing more siderophores as it competes more efficiently for iron and limiting the growth of pathogenic species (less competent) of the same organism at the same time (e.g. *Fusarium species*, *Aspergillus ochraceus*) (Aznar et al. 2014).

7.8.2 Virulence Factor

The siderophore-mediated transport system also plays an important role in microbial pathogenicity. Host pathogenic microorganism acquires iron to survive, and restricting its availability to others is considered to be an effective defence mechanism of host microbes that suggest that siderophores can act as virulence factors. For example, *Microbotryum violaceum* siderophore (mutant of plant pathogen) accumulates less rhodotorulic acid as compared to its wild type that exhibits minimal pathogenicity. On the other hand, mutant of *Ustilago maydis* (plant pathogen) showed defectiveness in siderophore production and is found to be as virulent as its wild-type strains. In addition, phototoxic and immunosuppressive effects also contribute to virulence in pathogenic organism in terms of chelating activity in siderophores. For example, *Alternaria cassiae* produces siderophore such as coprogen and ferricrocin which act as phytotoxins and several other siderophores such as desferrichrome which exhibits immunosuppressive effect in mouse model (Ecker et al. 2018).

7.8.3 Intracellular Iron Storage

Apart from solubilizing and transporting iron, siderophore also facilitates intracellular iron acquisition in mycelia and spores that effect germination. In case of *Neurospora crassa*, several intercellular siderophores produced by the species such as excretory coprogen and intracellular ferricrocin found in both hyphae and conidia are essential for its germination and iron storage. It is also well established that iron-siderophore complexes maintained in hyphae for the incorporation of spores help in sporulation and also transport iron to mycelial mitochondria. Several fungal species have both types of inter- and intracellular siderophores such examples *Penicillium chrysogenum*, *Aspergillus nidulans*, *Rhodotorula minuta* and *Ustilago sphaerogena* (Renshaw et al. 2002).

7.9 Applications of Siderophore

Siderophore and its different derivatives have broad scope in medical sciences and offer several applications in the field of biotechnology, microbial ecology and biomedical science. Various studies demonstrate the effective role of some siderophore in the management or treatment of human diseases and infections (Popat et al. 2017; Nagoba and Vedpathak 2011; Ahmed and Holmstrom 2014; Saha et al. 2016; De-Serrano 2017; Dimkpa 2016; Fine 2000; Banner and Woolf 2004). These are as listed in Table 7.4 which are as follows.

Table 7.4 Enlisting of biotechnological, ecological and biomedical applications of siderophores

Sl. no.	Area	Description
Biotechnological and ecological applications		
1.	Microbial ecology and taxonomy	The method of identification and characterization of microorganisms based on siderophore type they produce is known as 'siderotyping' which is further divided into analytical (HPLC analysis) and biological (specific DNA recognition) approaches
2.	Biocontrol of fish pathogens	Siderophores limit the iron availability in pathogenic fish (by making siderophore-iron complexes) which is necessary for the interaction in microbial virulence
3.	Optical biosensor	Pyoverdine has been investigated as an element of the sensor for molecular recognition in the identification of iron bioavailability in ocean water and soils

(continued)

Table 7.4 (continued)

Sl. no.	Area	Description
4.	Nuclear fuel reprocessing	Desferrioxamine B (hydroxamate and catecholate functionalities) is supposed to form strong complexes and have high-stability constants due to hard oxygen anions which help in separation of contaminants (acetanilide) during Purex process (fuel reprocessing)
5.	Bioremediation of environmental pollutant	Due to high affinity of siderophore towards metal ions such as Cd, Zn, Cu, Ni, and Pb, siderophores are extremely effective in solubilizing and increasing mobility of these metals and possess strong affinity to form stable complexes, thus making it useful in bioremediation
6.	Bio-bleaching of pulps	Siderophore (isolated from *Gloeophyllum trabeum*) effectively reduces the proportion of chemical used in bleaching process and can be an environment-friendly approach
7.	Siderophore promotes plant growth	Siderophores provide nutrition in form of iron to support their growth when the bioavailability of iron is low
8.	Soil mineral weathering	Siderophores help in protecting microbes against environmental stress by adhering to mineral surfaces
9.	Biogeochemical cycling of iron in the ocean	Siderophores make the iron available to phytoplankton in marine waters which plays an important role in their growth
Biomedical applications		
10.	Selective drug delivery-Trojan Horse strategy	Development of siderophore-antibiotic conjugates called 'sideromycins'
11.	Treatment of disease condition with iron overloading	Effective in conditions like haemochromatosis, sickle cell disease and thalassemia (major)
12.	Management of Malaria	Siderophore (such as desferrioxamine B) produced by *Streptomyces pilosus* has activity against *P. falciparum* (in vitro and in vivo)
13.	Removal of overload of transuranic elements like aluminium and vanadium	Siderophore named Desferal interacts with aluminium and vanadium ions and forms complexes which are readily excreted from the body via urine and faeces
14.	Cancer therapy	Several siderophores such as dexrazoxane, O-trensox, desferriexochelins and desferrithiocin are found to be effective in cancer therapy
15.	Antidote for iron poisoning	Deferoxamine B is used as antidote in acute iron poisoning and high iron loading conditions such as thalassaemia major and aluminium poisoning in chronic renal dialysis. Deferoxamine B is considered to have a high binding affinity for ferric form of iron that helps in removing excess iron from tissues and circulatory system

7.10   Conclusion

From recent investigations, it can be concluded that siderophores are the key components in iron transport in phototrophs and microorganisms. Structural variation and ligand specificity in siderophore as well as membrane receptors regulate the iron uptake process; hence, this field has immense potential for further exploration in the field of biomolecular science. The siderophore plays significant role in environmental applications and is also investigated as potential strategy in the field of biotechnology (agriculture, bioremediation and biosensor) and medicines (diagnosis and treatment). In a search of advance-level revelations, the metagenomic approach with detailed chemical examinations may be employed to improve the current environmental applications that also give new realm of investigation for siderophores. With the metal-chelating ability, siderophores are known to have potential applications in the field of medicine and biotechnology. Apart from iron binding (Fe^{3+}), the variety of siderophore is also investigated for binding with other metals including Pb^{2+}, Cr^{3+}, Al^{3+} and actinide ions. The study of metal-microbe conjugation highlights the significance of microbes which provides suitable environment for growth and reproduction of various forms of life. From the established literature, it is clear that siderophores represent the vital organic compounds for iron uptake among microbial and plant species. Siderophore variability in terms of their structural and functional characteristics and membrane receptors involving metal coordination in relation to microbial communities should be thoroughly investigated to establish the role of siderophore at profound level in the field of advance-level therapy in medical science.

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