Marine-derived Fungal Siderophores: A Perception

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Siderophores play crucial role in biogeochemical cycle in terrestrial as well as marine environment. Siderophores of bacteria from marine habitats have been extensively studied, however, comparatively less information is available on their fungal counterparts. This review focuses on siderophores of marine-derived fungi, molecular mechanism of siderophore biosynthesis and their uptake. Their chemical nature and applications are also discussed. Data so far available on marine fungal siderophores are found to be very interesting. Though less explored, the information available reveals novelty in chemical nature of siderophores of marine-derived fungi, i.e. occurrence of catecholates in fungi and carboxylates in non-mucoraceous fungi, which is the first-ever report. Further investigations on marine-derived fungal siderophores would be an interesting area of research.

[Key words: Siderophore, Marine-derived fungi, Catecholate, Carboxylate, Hydroxamate]

Introduction

Iron is an essential macronutrient for the growth of microorganisms. It plays crucial role in various cellular processes like DNA/RNA synthesis, ATP synthesis, respiration and also as a cofactor of numerous enzymes [1]. Despite its imperative role in microbial growth and being bountiful in nature, iron is not effortlessly available to microbes due to formation of ferric oxyhydroxide at neutral to alkaline pH in which the earth abounds[2, 3]. To combat this stress microbes (mostly aerobic bacteria, fungi and others) have developed a strategy i.e. chelation through production of siderophores. Siderophores are low molecular weight compounds with the strongest affinity for Fe$^{3+}$[4, 5]. They facilitate the conversion of Fe$^{3+}$ to Fe$^{2+}$ and make it available to microbes [6]. The term ‘siderophore’ had been coined from Greek word meaning “iron bearer”. The first siderophore discovered was ferrichrome produced by Ustilago sphaerogena which can work as growth factor for others [7]. Siderophores have gained interest in medical science due to its iron chelating efficiency for example, Deferoxamine-B – a natural siderophore produced by Streptomyces pilosus is broadly used in iron overload conditions during repeated blood transfusions[8]. While focusing on marine habitat, large ocean regions are known as high nitrate low chlorophyll (HNLC) because of low chlorophyll content in photosynthetic microorganisms. This HNLC region contains low iron concentration affecting the primary production. Marine microorganisms affected by this critical situation cope up with this stressing condition by producing siderophores to gain iron in HNLC regions [9-19]. While much work is done on bacterial siderophores from marine habitats, fungal siderophores have been completely overlooked [19]. As siderophore production by fungi in marine habitats is an interesting yet under explored area, this article mainly focuses on siderophores produced by marine-derived fungi. Our laboratory had contributed some pioneering work in this field. Vala et al. (2000) [20] had reported their work on siderophores of facultative marine fungi. They had worked with thirteen strains out of which, ten produced siderophores which were recognized as hydroxamates and carboxylates. Rests of three strains were non siderophore producers. The absence of siderophores in marine environment may be due to diffusion which makes siderophore production an unpainful exercise for the isolates. Other reason could be replacement of ferredoxins by flavodoxin which requires Mn$^{2+}$ rather than Fe$^{3+}$ in some marine isolates. However, imperative need for iron-dependent respiratory chains for aerobic life cannot be overlooked hence, non-detection could also be a possibility than non-production [21-24]. In their study, carboxylates were reported in mucoraceous fungi as well as a non mucoraceous fungus Paecilomyces variotii. Others were found to produce hydroxamate siderophores. Baakza et al.,
had given comparative data on siderophore production of marine and terrestrial fungi. They had selected ten marine and ten terrestrial fungal isolates. Screening these fungal isolates for siderophore production revealed valuable information about abundance of these molecules in both the habitats. Amongst these, a marine fungus *Cunninghamella elegans* was found to produce maximum (1987.5 µg/mL) siderophores while terrestrial *C. elegans* produced 1248.75 µg/mL indicating that marine fungi are equally important source of siderophores though less exploited. Some marine-derived fungi proved to be even more efficient siderophore producers when compared with terrestrials. Vala et al., (2006)\cite{25} had reported data regarding aspergilli from two different habitats: five from marine and five from terrestrial. *Aspergillus versicolor*, a marine isolate synthesized the highest 182.5 µg/mL on the fifteenth day and the lowest producers also were marine *Aspergillus* sp. and *Aspergillus niger*. Moreover, aspergilli from both the habitats were producers of hydroxamate siderophores.

Looking to the importance of marine-derived fungal siderophores which are yet under explored, our laboratory has extended the work on their role in mangrove reclamation by examining thirty four fungal isolates associated with mangrove *Avicennia marina* along Gujarat, West Coast of India. Among these thirty four fungal isolates, thirty two produced siderophores, again confirming wide distribution of siderophore production potential among mangrove associated fungi (Data unpublished).

**Molecular mechanism of siderophore biosynthesis and uptake**

**Siderophore biosynthesis in fungi**

Genes involved in siderophore biosynthesis, uptake and iron regulation in fungi have been well documented by Haas (2003)\cite{26} and Haas et al., (2008)\cite{27}. Elucidation of several unexplored aspects has been pursued further by several workers. While most fungal siderophores are hydroxamates, hydroxylation of L-ornithine by ornithine-\(N^\alpha\)-monoxygenase to \(N^\alpha\)-hydroxy-L-ornithine is the first step of the biosynthesis pathway (Fig.1). The hydroxamate group in the second step is then formed by transferring an acyl group (from acyl-coenzymeA) to \(N^\delta\)-hydroxyornithine, resulting in \(N^\delta\)-acyl- \(N^\alpha\)-hydroxy-L-ornithine with different possible acyl groups. These units are covalently linked via ester or peptide bonds to linear or cyclic oligomeric iron chelators by nonribosomal peptide synthetases (NRPSs) in the third step and finally the NRPS products can be altered further by non-NRPS enzymes in order to yield siderophore diversity\cite{26,28,29}.

\[\text{Oximine} \rightarrow \text{SidA} \rightarrow \text{N}^\alpha\text{-hydroxynornithine} \rightarrow \text{N}^\alpha\text{-acetyl-CoA} \rightarrow \text{SidD} \rightarrow \text{TARF} \rightarrow \text{N}^\delta\text{-hydroxy-L-ornithine} \rightarrow \text{SidG} \rightarrow \text{Acyl-CoA} \rightarrow \text{SidI} \rightarrow \text{N}^\delta\text{-acyl-N}^\alpha\text{-hydroxyornithine} \rightarrow \text{SidJ} \rightarrow \text{SidL} \rightarrow \text{Glycine} \rightarrow \text{Seine} \rightarrow \text{Ferricin} \rightarrow \text{Ferricin}

[Fig 1. Biosynthetic pathway of siderophores from *A. fumigatus* and *A. nidulans* (Adapted from Gründlinger et al., (2013a)\cite{30})]

Blatzer et al., (2011)\cite{30} identified and characterized *sidL* (encoding \(N^\delta\)-hydroxyornithine:acetyl coenzyme A (CoA)-\(N^\alpha\)-transacylase), the first siderophore biosynthetic gene not to be regulated by iron availability. They reported that the *sidL* is not genomically clustered with other siderophore-biosynthetic genes.

Recently, Gründlinger et al., (2013a)\cite{31} identified a new component (SidJ) of siderophore metabolism in *Aspergillus fumigatus* and reported its role in intracellular siderophore hydrolysis. The gene encoding SidJ was found to be localized in siderophore biosynthesis gene cluster. Furhter, Gründlinger et al., (2013b)\cite{32} demonstrated partial localization of fungal siderophore biosynthesis in peroxisome and reported the compartmentalization to be an evolutionary conserved region. Using green fluorescent protein-tagging, the authors ascertained peroxisomal localization of SidI (Mevalonyl-CoA
ligase), SidH (Mevalonyl-CoA hydratase) and SidF (anhydromevalonol-CoA transferase) in *A. fumigatus*. They suggested that as long as SidI, SidH and SidF share the same compartment, efficient siderophore biosynthesis can be achieved in both peroxisome and the cytosol. Johnson et al., (2013) [33] examined siderophore biosynthesis in foliar grass endophytic fungus *Epichloë festucae* and identified the role of sidN (encoding siderophore synthetase) in biosynthesis of extracellular siderophore epichloënin A which is related to ferrirubin of the ferrichrome family. Yasmin et al., (2012) [34] demonstrated a link between siderophore triacetylfusarinine C (TAFC) and ergosterol biosynthetic pathways. They identified sidI and sidH encoding acyl-CoA ligase and enoyl-CoA hydratase, respectively, in *A. fumigatus*. Initially while working with *Fusarium cubense*, Anke and Diekmann (1974) [35] proposed the role of such enzymes in biosynthesis of fusarinine-type siderophores.

While much of the information on siderophore biosynthesis is available for aspergilli, Lehner et al., (2013) [29] examined ten wild type strains of *Trichoderma* and found high degree of diversity among siderophores. They suggested further modifications of the nonribosomal peptide synthetase (NRPS) products to be responsible for the high diversity. They proposed that the fusarinine-type siderophore biosynthesis by *Trichoderma* is similar to well-understood process in *Aspergillus* sp.

Not only the genes involved in siderophore metabolism have been identified and characterized, but the supply of substrate for siderophore biosynthesis from different organelles has also been studied recently. Beckmann et al., (2013) [36] have reported the role of mitochondrial ornithine rather than cytosolic ornithine in siderophore biosynthesis in *A. fumigatus*.

**Transport of iron-siderophore complex in fungi**

Microorganisms depend on receptors present in various compartments of the cell envelope for siderophore uptake. There are four different mechanisms for siderophore mediated Fe³⁺ transport in fungi [37,38] as depicted in Fig. 2. Hajela (2010) [39] had studied the expression of siderophore mediated iron transport in fungi. Fungi generally take-up siderophore-iron chelates through transporters of unknown major facilitator (UMF)/ siderophore iron transporter (SIT) subfamily [27, 43, 44]. The siderophore transporters are one of the seventeen protein families exclusively found in fungi. Most of the information about siderophore-iron transporter in fungi is from the yeast Saccharomyces cerevisiae, which does not synthesize siderophores but can take-up xenosiderophores using membrane siderophore-iron transporters [45]. *A. fumigatus* has been predicted to encode ten siderophore iron transporters [46]. MirA, MirB, and MirC have been identified as siderophore transporters in *A. nidulans*, the expression of which is regulated by the transcription factors SreA and HapX [45, 47-49].

MirB was hypothesized to mediate siderophore uptake in *A. fumigatus* by Haas et al., (2008) [27] later Raymond-Bouchard et al., (2012) [45] pursued the siderophore-iron uptake by the transporter MirB from the same fungus in detail and reported its ability to transport siderophores coprogen, ferricrocin and TAFC as well.
Though molecular details of siderophore metabolism in fungi is being given due attention, the case is all the more critical for marine-derived fungi. Several fungi from marine environment have been observed to produce siderophores [19, 20, 25, 50]. However, scant information exists about molecular details of siderophores of this group of fungi [51].

**Status of siderophore biosynthesis in marine-derived fungi**

Wang et al., (2009) [52] purified and identified the siderophore of a marine-derived yeast *Aureobasidium pullulans* HN6.2 as fusigen having strong antibacterial activity against *Vibrio anguillarum*. They observed expression of gene encoding ornithine N5-oxgenase (catalyzing first committed step in siderophore biosynthesis) at transcription level and reported that the gene was repressed and enhanced in presence of iron and ornithine, respectively.

Recently, Chi et al., (2012) [51] cloned and characterized *sidA* gene (L-Ornithine-N5-Monoxygenase structural gene) from a marine-derived yeast *A. pullulans* HN6.2 and established physiological role of siderophore in marine-derived yeasts. The cloned gene was found to be 1461 bp encoding 486 amino acid protein with molecular weight 55.4kDa. The authors observed a negative effect of deleting the gene encoding L-ornithine-N5-monoxygenase on asexual reproduction of the test organism.

Further, Chi et al., (2013) [51] isolated and characterized the Sre1 (GATA Type transcription repressor) and studied the effect of gene disruption on biosynthesis of siderophores and expression of *sidA* gene. The expression of *sidA* gene was greatly de-repressed at transcription level when the disruptant (R6) was grown in iron-repleted medium. The authors are still exploring how does the Sre1 bind to the promoter of *sidA* gene, as the promoter for *sidA* contains the sequence 5'-HGATAR-3' and not the sequence 5'-ATCWGATAA-3' [51]. Haas et al., (1999) [54] reported derepression of L-ornithine-N5-oxgenase activity and consequently derepression of the biosynthesis of the hydroxamate siderophore N,N',N''-triacetyllysarazine in *A. nidulans* when supplied with sufficient iron concentration.

**Screening and Detection of siderophores**

Screening procedures for siderophore production by terrestrial and marine isolates include FeCl₃ test [55], Chrome Azurol S (CAS) assay and CAS agar plate test [56]. In case of detection of siderophores from marine-derived fungi, we have observed [Fig. 3] that modified CAS agar [57] suited better. CAS agar plate contains Hexadecyltrimethylammonium (HDTMA), a detergent that is toxic to growth of the cells. Hence, siderophore production is inhibited. In modified CAS agar plate, half of the plate contains CAS and half growth medium.

As only half of the plate contains CAS, HDTMA does not hinder the growth of the fungus. Thus, siderophores appears as orange-red zones around the fungal growth [Fig. 3].

![Image 3](https://via.placeholder.com/150)

**Chemical Nature of Siderophores**

Siderophores are chemically categorized according to the functional group used to coordinate Fe³⁺. Siderophores are mainly characterized as hydroxamates, catecholates, carboxylates, mixed type siderophores and a suite of amphiphilic siderophores (produced exclusively by marine bacteria). Chemical nature of siderophores can be detected by chemical as well as bioassays. Bioassays are more sensitive than chemical assays which include the use of transport defective indicator strains.

Hydroxamate siderophores are known to be produced by bacteria and by most fungi [58, 59]. Hydroxamate nature of siderophore is confirmed by FeCl₃ test [60] showing λ_max between 420-450 nm and tetrazolium salt test [61]. Bioassay for hydroxamate is carried out by using *Arthrobacter flavescens* JG9 [62]. Marine aspergilli like, *Aspergillus* sp., *A. versicolor* and *A. niger* are also known to produce hydroxamate siderophores [25]. Wang et al., (2009) [52] have reported marine-derived *A. pullulans* to produce siderophore fusigen, a type of hydroxamate siderophore (Fig. 4).
Hydroxamate siderophores are further categorized as mono-, di- and tri-hydroxamates, based on number of hydroxamate groups. Based on the number of bonds the ligand forms with metal ion, ligand denticity identifies them as bi-, tetra- and hexadentates. Vala et al., (2006) observed occurrence of trihydroxamate hexadentate siderophores in marine as well as terrestrial environment. A higher stability constant of hexadentates is the reason behind their predominance over bi- and tetradenates in both marine and terrestrial environment. In our recent study, out of thirty two isolates, thirty one belonging to Ascomycota produced hydroxamate siderophores as expected. Among these thirty one isolates, twenty seven produced trihydroxamate hexadentates whereas four produced tetradentates which is a novel behavior. 

Catecholate nature of siderophore can be detected by FeCl₃ test showing λₘₐₓ at 495 nm, Arnow’s test and bioassay by using Salmonella typhimurium enb.⁷ [62]. Catecholate siderophores having catechol as functional group were initially reported to be produced only by bacteria. One of the marine fungal strains of Penicillium bilaii exhibited a novel behavior. This strain produced pistillar in siderophore which is chemically a catecholate when grown under relatively high iron concentrations. The structure of pistillar is as shown in (Fig. 5).

In our recent study (data unpublished) one of the strains yet to be identified has also shown production of catecholate and hydroxamate siderophores. Further structural characterization of this siderophore is in progress in our laboratory.

Amphiphilic siderophores have one or two series of fatty acid attached with head group which co ordinates with Fe²⁺. Amphiphilic siderophore like amphibactins (Vibrio sp.18) and ochrobactins (Ochrobactrum sp.18) are hydrophobic in nature while loihichelins (Halomonas sp. LOB5) are hydrophilic. Amphiphilicity is based on variations in composition of head group relative to fatty acid chain length. To the best of our knowledge so far amphiphilic siderophores have not been reported in marine-derived fungi. It was normally believed that all fungal siderophores were hydroxamates but then Winkelmann (1991) proposed a new siderophore from Rhizopus microsporus showing the presence of citric acid and named it rhizoferrin. Carboxylates are exclusively produced by fungi belonging to Mucorales and a few bacteria. They are detected by spectrophotometric analysis [68] and bioassay using Morganella morganii. One of the strains of marine-derived P. variotii also produced carboxylate siderophore and hence exhibited occurrence of carboxylate outside Mucorales. The siderophore produced by C. elegans ATCC36112 was identified and confirmed as rhizoferrin by using one and two dimensional NMR [19]. The authors compared the observed data with published data for the identification and confirmation of rhizoferrin. A possible role of marine fungal siderophores in hydrocarbon degradation, a pollutant extensively contaminating marine environment is under process in our laboratory.

Mixed types of siderophores show great diversity of structures as in fluorescent pseudomonads that produce pyoverdines exemplified by extracellular production of yellow-green fluorescent pigment. Structures of only few of the siderophores from marine-derived fungal isolates have been characterized and analyzed. Further investigations in this line could be an interesting area of research.

**Applications**

Siderophore production is not restricted to laboratory conditions but also found in soil and aquatic conditions. Hence, they have significant
ecological as well as economical roles. Some of them are described below.

Selective utilization of siderophores in mixed microbial populations is a key mechanism for preventing emergence of unwanted competing organisms.

There are various applications of siderophores reported in medical field. When siderophores bind with antibiotics, they form sideromycins, a new way for drug delivery. Artemisinin, an antimalarial agent combines with analogue of Mycobactin expressing antituberculosis activity in *M. tuberculosis* [72]. Siderophore produced by *Klebsiella pneumoniae* had been used as antimalarial agent [73], and also as deodorants [74]. Deferoxamine well known for its use in iron overload conditions has also been used in removal of vanadium [75].

The medical applications of siderophores of marine origin are yet to be explored.

Siderophores also play an important role in agriculture. *Pseudomonas putida* produces pseudobactin which enhances plant growth when soil is inoculated with these organisms [76]. Systemic resistance is thought to be induced by siderophores in plants causing disease suppression [77]. Siderophores produced by bacteria with higher chelating efficiency makes iron availability restricted for pathogens and lets the bacteria succeed in competition [78, 79]. Study on role of siderophores of marine-derived fungi in biocontrol of plant pathogens is in progress in our laboratory.

Pyoverdine produced by fluorescent pseudomonads could be an important tool to diversify closely related strains [80, 81]. Strains having identical pyoverdine show same iso electrical focusing (IEF). On other hand different pyoverdines exhibit different IEF patterns. Based on the patterns exhibited by siderophores in IEF, siderotyping involves separation of siderophores based on their iso electric pH.

*Marinobacter hydrocarbonoclasticus*, an oil degrading marine bacterium isolated by Barbeau et al., (2003) [82] have been reported to produce petrobactin. It has been suggested that hydrocarbons can be degraded by iron facilitation in bacteria present in marine ecosystems.

Soil contaminated with heavy metals is a major problem that warrants attention for bioremediation [83]. While some of the conventional methods used prove unsuitable economically and also result in degradation of soil texture [84], application of siderophores for bioremediation of metal contamination could be a promising alternative. In metal contaminated soil that is iron deficient, microorganisms producing siderophores reduce the metal load as they can chelate other metals like nickel also besides iron.

Siderophores are known to play a significant role in cellulose depolymerization and lignin modification. It may be possible to function with Mn as the catalyst for depolymerization process which could open the wood structure to allow penetration of large enzyme because of low molecular weight of siderophore-metal complexes and oxidizing potential of the transition metals. Siderophores are known to scavenge transition metals for extracellular enzyme production and fungal metabolism [85].

It has been reported recently [86] that dissolution of spent nuclear fuel is influenced even by low concentration of siderophores, be it synthetic deferoxamine or pyoverdines synthesized by *Pseudomonas fluorescens*. Siderophores are proposed to play a role in radioactive waste remediation and nuclear fuel reprocessing [38].

Exceptional Fe$^{3+}$ binding constant of siderophores make them an ideal selection for molecular recognition element and they could be used for detecting Fe-bioavailability in soils or oceanic water [87]. Pesce and Kaplan 1990 [88] had proposed pyoverdines as suitable candidates for construction of biosensor.

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