

## Review

Luis O. De Serrano\*

# Biotechnology of siderophores in high-impact scientific fields

<https://doi.org/10.1515/bmc-2017-0016>

Received April 10, 2017; accepted June 29, 2017

**Abstract:** Different aspects of bacterial and fungal siderophore biotechnological applications will be discussed. Areas of application presented include, but are not limited to agriculture, medicine, pharmacology, bioremediation, biodegradation and food industry. In agriculture-related applications, siderophores could be employed to enhance plant growth due to their uptake by rhizobia. Siderophores hindered the presence of plant pathogens in biocontrol strategies. Bioremediation studies on siderophores discuss mostly the mobilization of heavy metals and radionuclides; the emulsifying effects of siderophore-producing microorganisms in oil-contaminated environments are also presented. The different applications found in literature based in medicine and pharmacological approaches range from iron overload to drug delivery systems and, more recently, vaccines. Additional research should be done in siderophore production and their metabolic relevance to have a deeper understanding for future biotechnological advances.

**Keywords:** biocontrol; bioremediation; drug delivery; nanotechnology; siderophores.

## Introduction

Iron plays an important function in different cellular processes like DNA metabolism, protein function, fatty acid synthesis and other chemical reactions. This importance is observed at all levels of the tree of life. However, in humans, iron concentration higher than normal levels could become toxic by increasing oxidative stress,

eventually translating to myocardial malfunction and inflammation responses to the pancreas (1). Other studies have suggested damages to adipose tissue and liver in obese persons (2). Additionally, iron is well known to be required for plant nutrition, development and health (3–5). Photosynthesis and chlorophyll biosynthesis are affected at low iron concentrations, hindering plant growth (6, 7).

The acquisition of iron in the bacterial cell is commonly regulated by the *fur* box (for ferric uptake regulation) in an iron concentration-dependent process (8). At high iron concentration siderophores are not produced due to binding of the Fur protein on the siderophore gene promoter. When low intracellular iron concentration occurs, a conformational change in the Fur protein causes its detachment from the siderophore gene promoter, re-establishing the transcription of the gene and the eventual synthesis of siderophores. Transport of iron-bound siderophores is mediated by membrane receptors, periplasmic membrane proteins and TonB-like transporters (9) and after internalization, ferric iron is reduced to its ferrous form. Additional iron acquisition systems include hemophores (10, 11) and heme (12, 13).

Because of the relevant effects of iron at different levels in the tree of life, scientists have studied the role of siderophores for a variety of biotechnology purposes. The agricultural, environmental, bioremediative, medical and food industry aspects of siderophore biotechnology will be discussed ahead and are summarized in Table 1. Fostering the studies in siderophore biosynthesis, production and characterization will eventually lead to novel approaches and applications, benefiting society in general.

## Plant growth and biocontrol of pathogens

Scientists have researched different aspects of iron and siderophore applications for agricultural purposes. The main hypothesis is on the natural potential of siderophore utilization to help concentrate the natural iron source

\*Corresponding author: Luis O. De Serrano, Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT 59812, USA; and Center for Translational Medicine, University of Montana, 32 Campus Dr., Missoula, MT 59801, USA, e-mail: [luis.deserrano@mso.umt.edu](mailto:luis.deserrano@mso.umt.edu).  
<http://orcid.org/0000-0003-2015-8107>

**Table 1:** Overview of siderophores discussed and applied in different high-impact scientific areas.

Siderophore or compound	Microorganism(s)	Biotechnological application(s)	Reference(s)
2,3-Dihydroxybenzoic acid	<i>Bacillus</i> sp.	Food industry	(14)
2,3-Dihydroxybenzoyl glycine	<i>Bacillus</i> sp.	Food industry	(15)
Aerobactin	<i>Escherichia coli</i>	Medicine	(16)
Aeruginosins	Cyanobacteria	Medicine	(17)
Arthrobactin	<i>Arthrobacter</i> sp.	Medicine	(18)
Bacillibactin	<i>Bacillus subtilis</i>	Agriculture	(19, 20)
Catecholate and hydroxamate siderophores	<i>Pseudomonas azotoformans</i> and marine bacteria	Bioremediation	(21, 22)
Coprogen	<i>Penicillium chrysogenum</i> , <i>Histoplasma capsulatum</i> , <i>Neurospora crassa</i> and <i>Penicillium nalgiovense</i>	Agriculture and medicine	(19, 23–25)
Deferriferrichrysin	<i>Aspergillus oryzae</i>	Food Industry	(26)
Desferrioxamine B	<i>Streptomyces pilosus</i>	Bioremediation, medicine and pharmacological	(27–30)
Dimerium acid	<i>P. chrysogenum</i> and <i>H. capsulatum</i>	Agriculture and medicine	(19, 23)
Ferrichrome	<i>P. chrysogenum</i>	Medicine	(25)
Ferricrocin	<i>P. chrysogenum</i>	Agriculture	(19)
Ferrioxamine E	<i>S. antibioticum</i>	Medicine	(31)
Fusarinine	<i>P. chrysogenum</i> and <i>H. capsulatum</i>	Agriculture and medicine	(19, 23)
Fusigen	<i>Aureobasidium pullulans</i>	Medicine	(32, 33)
Hydroxypyridinonate ligands	— <sup>a</sup>	Medicine	(34)
Mycobactin	<i>Mycobacterium tuberculosis</i>	Medicine	(35)
Neocoprogen	<i>P. nalgiovense</i>	Medicine	(24)
Ochrobactins	<i>Vibrio</i> sp.	Bioremediation	(36)
Petrobactin	<i>Marinobactin hydrocarbonoclasticus</i>	Bioremediation	(37, 38)
Pyochelin	<i>Pseudomonas aeruginosa</i>	Medicine	(33)
Pyoverdine	<i>Pseudomonas fluorescens</i>	Agriculture and bioremediation	(39, 40)
Sideromycins	<i>Streptomyces</i> sp.	Medicine	(32)
Triacetylfulsarinine	<i>Saccharomyces cerevisiae</i> and <i>P. nalgiovense</i>	Medicine	(24, 31)
Vanchrobactin	<i>Vibrio anguillarum</i>	Medicine	(41)
Vibriobactin	<i>Vibrio cholerae</i>	Medicine	(42)
Yersiniabactin	<i>Yersinia pestis</i>	Medicine	(42)

<sup>a</sup>Synthetic ferric iron ligands. Note that the ‘Biotechnological application’ column is presented but this does not limit future biotechnological applications of the siderophore.

found in soils. For example, endophytes use siderophores as a key component to improve plant iron uptake from soil and also help in the production of indole-3-acetic acid, which is a plant growth hormone (43). Bacterial endophytes from invasive *Sorghum* sp. changed the rhizobial population of native plants allowing the former to improve its survival rate. Also it was noticed that horizontal transfer of the rhizome population occurred. Simple plants, as bryophytes (moss) from the species *Racomitrium japonicum*, benefited from their rhizobia due to inhibition of pathogenic fungi and plant growth enhancement (44). Researchers in this study identified three major species that were involved in moss rhizobia composition: *Pseudomonas*, *Rhodococcus* and *Duganella*. Rhizobia siderophore production was confirmed in the study. The potential use of rhizobia as biofertilizers was studied, presenting a good way to improve moss growth and create an industry for green roofs.

There are siderophores that, in a clinical setting, can be detrimental to the host in bacterial infections but in other settings could potentially be beneficial. An example of this statement is the production of the siderophore pyoverdine by *Pseudomonas fluorescens* (39), which is related to virulence and pathogenicity of the microbe at the clinical level (45–47). Nagata and co-workers (39) found that pyoverdine production enhanced iron nutrition of tomato plants (Strategy I plants, which cannot produce pyoverdine). Strategy I plants use proton extrusion to increase their ferric iron uptake. When researchers compared ferrous iron and pyoverdine supplementation a significant accumulation of ferric iron in roots and leaves was detected. Also, plants showed better growth when supplemented with ferric iron and the siderophore than with just ferric iron alone, demonstrated by an increase in leaves and plant weight. Molecular biology tests for ferric-chelate reductase and iron-regulated transporter genes

showed decreased expression. Pigment (chlorophyll, carotenoids and anthocyanin) production was restored to levels of ferrous iron-treated plants. Thus, *Pseudomonas*-produced pyoverdine could potentially be used as a biofertilizer component. Another report examined siderophore production and its application on wheat harvest (48). Several strains (43) of siderophore producer microorganisms were evaluated for biocontrol purposes against *Rhizoctonia solani*, a plant fungal pathogen that develops in both cultured and non-cultured soils. It infects mostly seeds and during plant growth causes damping-off and root rot. From the 14 isolates studied, Mst 8.2 showed more inhibition of the fungal pathogen radial growth by two different assays (round circle and spot inoculation assays), reducing it by 41% and 71%, respectively. The strain also produced several antibiotics, chitinase and protease, essential metabolites that play a role in its antifungal properties. Wheat plants seeds treated with Mst 8.2 bacterial suspensions showed root rot lesion reduction and enhanced plant growth. After 16S rRNA sequencing, strain Mst 8.2 was found to be related to *P. fluorescens*. Another rhizobacterium, *Alcaligenes faecalis*, associated with the groundnut rhizosphere was found to produce siderophores (49). Inoculation of this microorganism enhanced several plant growth characteristics, like seed germination, root length, shoot length and chlorophyll content.

Nabti and co-workers (50) studied the beneficial rhizobacterium *Cellulosimicrobium* sp. and its barley growth-promoting traits. This bacterium produced several antibiotics, siderophores and enzymes necessary for soil colonization of plants. It inhibited growth of several fungal pathogens of barley, including: *Botrytis*, *Fusarium* and *Verticillium*. Therefore, it has potential both as an inoculant and biocontrol agent. Also some *Bacillus* species are found to be plant growth promoting bacteria (51). Root and plant weight improved in bacilli-treated wheat plants and siderophore-producing bacilli were the best performers. However, no siderophore mutants were prepared to confirm that siderophore production is linked with plant growth and health.

Fusarinines and dimerium acid produced by the fungus *Penicillium chrysogenum* are also involved in iron utilization of strategy I and II plants (19). The siderophore mixture increased the iron content (as chlorophyll concentration) in the plants studied (cucumber and maize). Other siderophores identified by mass spectrometry were coprogen and ferricrocin, confirming that dimerium acid and fusarinine are hydrolysis degradation products of coprogen. Plants showed robust growth when the siderophore mixture or the hydrolysates were provided at iron-deficient conditions. Recently, a team of researchers

studied the production of plant growth promoting factors from soil yeasts (20). No direct experiments correlating siderophore production and plant growth benefits were performed. We suggest further investigations towards the goal of structure determination and mechanistic effects of siderophore(s) on plant pathogens and plant growth.

Bacillibactin has been also studied as a siderophore with potential uses in agriculture as a biocontrol agent (52, 53). In the Yu and co-workers (52) study, siderophore-producing *Bacillus subtilis* inhibited *Fusarium* wilt disease and enhanced plant growth in peppers. However, iron supplementation helped the fungal pathogen in colonizing the plant even if the beneficial, siderophore-producing microbe was present. Also Woo and Kim showed that this siderophore can be a good biocontrol agent of the red pepper blight pathogen *Phytophthora capsici*. This demonstrates the potential use of bacillibactin as a biocontrol agent and an enhancer of plant growth. Siderophores enhanced iron solubilization and this was confirmed by a study with *Acinetobacter calcoaceticus* (54). The bacterium degrades the phosphate pesticide chlorpyrifos, helping in phosphorus solubilization available to the plant, and produces siderophores that enhance plant growth. Another example of biocontrol by siderophore production and iron availability and sequestration was studied on a strain isolated from the plant *Arisaema erubescens* (55). The strain was identified by molecular techniques as *Bacillus atropheus* and produced siderophores. Siderophore extracts prevented the growth of the cotton wilt pathogen *Fusarium oxysporum*. As the literature shows, several siderophores or siderophore-producing microorganisms could be implemented in biofertilizers, rhizobial inoculations, sprays and/or biocontrol agents to benefit important crops for human consumption.

## Food industry

There is little information available of how siderophores could be applied to the food industry. An important report connects together the production of a siderophore (deferriferrichrysin) from *Aspergillus oryzae* and the reduction of oxidation in food products (26). The researchers optimized deferriferrichrysin production to 2800 mg/l. The siderophore was stable at different temperature conditions. Deferriferrichrysin demonstrated antioxidant activity when compared to EDTA as the control iron chelator. Another seminal article presents the production, purification and characterization of a catecholate type siderophore (2,3-dihydroxybenzoic acid) produced by

a probiotic *Bacillus* spp. (14). The same research team studied the antimicrobial properties of the probiotic microorganism against different pathogenic bacteria, presenting positive results. In another article, 2,3-dihydroxybenzoic acid (56). However, siderophore production correlation with probiotic function was not establish indicating to us that further research is needed.

A third report presented a similar siderophore produced by a human-isolated *Bacillus* sp.: 2,3-dihydroxybenzoyl glycine (15). After production and purification experiments the siderophore was characterized by Fourier-transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and electrospray ionization-mass spectrometry (ESI-MS) confirming the structure to be similar to that of 2,3-dihydroxybenzoic acid. The probiotic microbe presented antimicrobial activity against several pathogenic bacteria but no siderophore mutants were generated to assess if siderophores are the responsible metabolites for such behavior. It is imperative for researchers to employ experiments that will determine if siderophore production is linked to beneficial probiotic bacteria, creating a novel application of these metabolites in the food industry.

## Bioremediation and biodegradation applications

Bioremediation is the utilization of organisms to clean-up contaminated sites, like soil, water, oceans, etc. Several heavy metal contaminants including lead (Pb), nickel, copper, cadmium, zinc and chromium (Cr) can be found in soil and sediments from bodies of water (57). Other heavy metals present in diverse contaminated sites include arsenic, strontium and titanium (58, 59). Most of these heavy metals are detrimental to living organisms but bacterial community analysis has shown that microorganisms can grow in the presence of these contaminants (21, 60). Other bacterial species tend to grow in environments contaminated with petroleum-based oils or radionuclides (22, 40, 61). Some examples of bioremediation of heavy metals and radionuclide mobilization by siderophore and siderophore-producing microorganisms will be discussed below.

Mines are the ideal sites to isolate strains that will have the potential of metal transformations. A particular example is the study of copper mines and how the extracted ore could be used as the sole energy source for isolate growth (62). The Lubin copper mine was studied and isolates from different genera (*Microbacterium*, *Acinetobacter*, *Bacillus* and *Pseudomonas*) were obtained from the ore. Multi-resistance to copper, arsenic, nickel

and zinc was confirmed in the study. In another report, Nair and co-workers found a direct role of siderophores in arsenic soil decontamination (27). The siderophore was produced by *Pseudomonas azotoformans* and FTIR studies revealed hydrogen bonding formation between the siderophore and arsenic. Arsenic removal by the molecule was 92.8% compared to citric acid and EDTA (70% and 77.3%, respectively). The study did not observe for concentration-wise removal characteristics that will reveal more information about the process and the potential of siderophores as bioremediation agents. However, bioremediation by siderophores, or siderophore-producing microorganisms, may be more complex. A study using siderophore producing *Pseudomonas aeruginosa*, *P. fluorescens* and *Ralstonia metallidurans* showed that rhizobial inoculation with these species to maize plants increased the phytoremediation of Cr and Pb (37). Siderophore production and cell growth increased with the incorporation of skim milk and calcium alginate beads in the growth media. Another microorganism, *Staphylococcus arlettae*, also is responsible for Cr (VI) reduction and its bioremediation from industrial effluent samples (38). Cr removal was at 98% in 120 h and plant growth effects were tested on common wheat plants showing shoot and root length improvement.

Radionuclides present a contamination problem especially with industrial, mining, weapons testing and nuclear energy demand and related accidents or anthropogenic activities (like Chernobyl and Fukushima). There is evidence of radionuclide immobilization by microorganisms in the available literature (22, 36, 40). As demonstrated in the literature and previous research, siderophores can be used as bioremediation agents to immobilize radionuclides and heavy metals. The containment or removal results may vary due to concentration of siderophore, solubility, bioavailability of the metal or radionuclide and several environmental conditions like pH, electron potential and ionic strength (40). In brief, a radionuclide phase (water soluble or insoluble) is changed by microbial metabolism (biotransformation), avoiding further contamination of the ecosystem. Uranium removal from mine ore was assessed in a study published by Edberg and co-workers (36). They noticed that pyoverdine from *P. fluorescens* has the potential to reduce uranium and decrease its water solubility. Iron solubility was also reduced but this could be also done in an abiotic approach by the formation of iron oxides  $[\text{Fe}(\text{OH})_3]$ . Farkas and colleagues (22) determined that some bacterial isolates from a mine to be considered for a nuclear waste repository were siderophore producers and radiotolerant. Polonium (Po) and protactinium (Pa) have been found to be chelated by hydroxamic siderophores from marine bacteria (63).



Other researchers proposed the use of siderophores for plutonium phytoremediation at the Savannah River site (64). Another radionuclide studied by researchers was uranium and its immobilization by desferrioxamine B, a medically relevant siderophore (28). In brief, uranyl was desorbed from kaolinite when the siderophore concentration was about 100  $\mu\text{M}$ . This effect was not only due to desorption capabilities of desferrioxamine B, but also by neutral species formed during the process.

Some evidence of petroleum-degrading bacteria and siderophore production has been published (29, 65). Barbeau and colleagues (29) isolated the petroleum-degrading bacterium *Marinobacter hydrocarbonoclasticus* and studied its siderophore: petrobactin. No studies on how siderophore affected oil-degrading capabilities or mechanisms were done but it was inferred that this petrobactin helps in iron acquisition from the petroleum components. Other oil-degrading species like *Vibrio* sp. isolated from the Gulf of Mexico (after the Deep Water Horizon oil spill) produced siderophores (30). The ochrobactins may be involved in the metabolic characteristics of this petroleum-degrading microorganism. Lin and co-workers isolated an oil-degrading bacteria, *Pseudomonas sagittaria*, from contaminated soil, that produced siderophores (61). From the literature reviewed, no structural studies were performed to determine the type of siderophore functional groups present. Additionally, no studies correlated siderophore production with oil-degrading activity. Although there is no direct evidence of petroleum degradation by siderophore-producing bacteria, this is suggested by some approaches and more studies should aim to elucidate the relationship at bioremediation levels.

## Pharmacological and medical applications

Metabolites produced by bacteria, or microorganisms, have the potential to serve as pharmacological targets (biomarkers) or have therapeutic effects. During this section we will focus mainly in how siderophores can be used or applied to treat different diseases or help to develop diagnostic tests to improve human health, among other approaches. The first and most studied aspect of siderophore biotechnology is the treatment of iron overload during transfusion due to non-hemorrhagic conditions (34, 66). Desferrioxamine B, a siderophore produced by the bacteria *Streptomyces pilosus*, has been used in different cases of iron poisoning, or overload, confirming its biotechnological potential (17). Deferrrioxamine chelate

both ferric ( $\text{Fe}^{3+}$ ) and ferrous ( $\text{Fe}^{2+}$ ) iron (67). Bergeron and colleagues modified the desferrioxamine structure starting from the premise that a low molecular weight molecule could function better as a pharmacophore (68). The structural modification caused a differentiation in the lipophilicity of the molecule when compared to the parent molecule chelator. Other synthetic heteropodate iron and metal chelators have been developed which eventually could have medical applications (18).

Antimicrobial properties of siderophore-producing microorganisms have been previously described. One of the first key studies published presented *Azospirillum lipoferum* to produce siderophores (35). This microbe associated with different plant rhizospheres, promoting nitrogen fixation. To determine siderophore antimicrobial properties, extracts were tested against different bacterial and fungal species. Bactericidal action was observed in *Rhizobium* species, *Azotobacter*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus* and *Bacillus* in cowpea. Fungicidal activity was detected in *Rhizoctonia*, *Fusarium oxysporium*, *Aspergillus niger*, *Aspergillus sydowii*, *Aspergillus varicolor* and *Penicillium funiculosum*. In a similar study, isolates (258) from two 'cenotes' environmental samples were studied for interspecies interactions and antimicrobial properties (69). A representative fraction of the isolates (13%) showed antimicrobial properties due to siderophore production. Most of the antimicrobially active isolates were identified as members of the genera *Aeromonas*, *Bacillus*, *Burkholderia*, *Photobacterium*, *Pseudomonas*, *Serratia*, *Shewanella* and *Stenotrophomonas*. Some microorganisms, like cyanobacteria, from either terrestrial or freshwater environments have been found to produce siderophores with potential biotechnological applications (70). The study showed how extracts from cyanobacteria isolates that produced siderophores (aeruginosins) have antimicrobial properties. Also other toxins and antimicrobial agents were produced by these microbes, including microcystin and cyanopeptolin. Antimicrobial properties were described also in *Stenotrophomonas maltophilia*, identified as a siderophore-producing bacteria (71). This microorganism has become very important for biotechnological purposes and also for its increasing role in nosocomial infections. Researchers studied 50 environmental and clinical *S. maltophilia* isolates and their inter-species interaction. Antifungal activities against the human pathogen *Candida albicans* were detected more on environmental isolates which produced siderophores and antibiotics.

Another siderophore presenting antimicrobial properties is fusigen, produced by the yeast *Aureobasidium pullulans* (32, 33). Wang et al. (33) presented the siderophore

screening of 300 yeast species confirming the production of fusigen by the previously mentioned *A. pullulans*. The antimicrobial activity of fusigen were confirmed in the marine pathogens *Vibrio anguillarum* and *Vibrio parahaemolyticus*. In a following report, investigators disrupted the *sidA* gene encoding for the siderophore and the yeast did not show antimicrobial activity against the previously mentioned marine pathogens (32). Recent genome sequencing studies showed that *A. pullulans* contains certain clusters of non-ribosomal peptide synthetases (NRPS) (41), which are enzymes involved in siderophore biosynthetic pathways (8, 72).

Another application for siderophore biotechnology is antimicrobial conjugates. In this application the siderophore of interest is attached to an antibiotic and used as a 'Trojan horse' to serve as a drug delivery agent (73–75). This approach facilitates antibiotic transport throughout the membrane using siderophore receptors. Two arthrobactin-carbacephem conjugates were developed and *E. coli* was treated at 10  $\mu\text{M}$  of the conjugate (73). Bacterial growth was affected by the siderophore-antibiotic conjugates and the effect was enhanced if an iron chelator (like EDDA) was added to the growth media. Siderophore- $\beta$ -lactam conjugates have also been of interest (16). Cephalosporin conjugated to a siderophore was tested against different pathogenic bacteria including *E. coli*, *P. aeruginosa* and *S. aureus*. Artificial siderophores have been developed and their antimicrobial properties have been studied when conjugated with a carbacephalosporin (Lorabid) (76). Treating simultaneously with hydroxamic- and catechol-containing siderophore conjugates decreased bacterial growth. Other molecules of interest are the sideromycins, linked with either Lorabid or ciprofloxacin (42). The first conjugate attacks the periplasm and the later one the cell wall. The sideromycins effectively inhibited growth of *S. aureus*. Another siderophore conjugate complex studied was pyochelin-norfloxacin (77). Analogues of the siderophore were synthesized and linked to norfloxacin (antibiotic). Antimicrobial activities were tested on *Pseudomonas* and two of the four conjugates were effective against the pathogen. Another example of siderophore analogs is the vanchrobactin-norfloxacin conjugate which showed antimicrobial activities against *V. anguillarum* and its mutants (78). However, there is evidence that some siderophore-antibiotic conjugates improve bacterial growth. Spermexatol-carbacephalosporin conjugates showed no effects on *Mycobacterium smegmatis* (79). A common situation found during clinical development of antimicrobials is when *in vivo* and *in vitro* studies do not correlate, making the solution process cumbersome. An example of this situation is a siderophore-monobactam compound, MB-1, that was

studied for its potential in *P. aeruginosa* growth inhibition (31). *In vitro* and *in vivo* assays did not correlated may be due to native siderophore competitors that allowed the bacteria to survive in *in vivo* studies.

Siderophores have been used to develop vaccines and have been covered extensively (80–84). Bergeron and colleagues reported the use of a vibriobactin analogue linked to either ovalbumin (OVA) or bovine serum albumin (BSA) and their ability to promote antibody production in mice (85). The immune response was confirmed by the detection of anti-BSA and -OVA IgG isolated from mice plasma. Recently a group of researchers focused their attention on siderophore-antigen conjugates to develop vaccines for the treatment of urinary tract infections (UTIs) based on an uropathogenic *E. coli* (UPEC) murine model (83). Mike and co-workers designed and produced yersiniabactin (Ybt) and aerobactin-cationized bovine serum albumin (Aer-cBSA) conjugates and evaluated their vaccination applicability. Researchers found a reduction in bacterial burden when mice were treated with siderophore-cBSA conjugates, including a 1:1 Ybt-cBSA:Aer-cBSA mix of conjugates, suggesting protection by adaptive immune responses. Other reports showed the use of iron transport receptors in bacterial pathogens as important components of vaccines (86). Siderophore receptors and porin proteins from *E. coli* O157:H7 were evaluated on cattle (87). Two and 3 ml doses of the vaccine were injected subcutaneously and prevalence of *E. coli* O157:H7 was reduced in both treatments, but in contrast the 3 ml dose obtained fewer days of the cattle being positive for *E. coli*. In another case, the FhuD receptor of *S. aureus* was analyzed as a potential vaccine component (88). FhuD-ferrichrome was used as the model vaccine antigen and was determined to have potential due to the lack of conformational changes.

A report by Howard and co-workers (23) studied the production of siderophores from *Histoplasma capsulatum* and their potential uses as antibodies. After mass spectrometry analysis five hydroxamic siderophores were identified, including dimerium acid, acetyl dimerium acid, coprogen B, methyl coprogen B and fusarinine. Investigators formulated a BSA-dimerium acid polyclonal antiserum administered to a rabbit. Dimerium acid and the other siderophores were then conjugated to keyhole limpet hemocyanine (KLH) to prepare the test antigens, with successful detection of anti-siderophore antibodies for all types of siderophores studied.

There is also the potential to use siderophores as radiopharmaceutical imaging agents. Petrik and colleagues demonstrated clinical use in imaging pulmonary aspergillosis (89). Mouse and rat infection models were developed and siderophores (triacetylfusarinine and ferrioxamine E)

were labeled with gallium 68 ( $^{68}\text{Ga}$ ). In both models with iron-deficient conditions, *Aspergillus fumigatus* absorbed both of the siderophore-radionuclide conjugates. There was rapid excretion of the labeled siderophores by the renal system in normal mice. Conjugate accumulation in the lungs was observed in the rat model by positron emission tomography (PET) and increased with the severity of infection (correlating with CT images). An important fact that was not discussed in this paper is how fungal growth could be affected by these conjugates at spatio-temporal scales.

Other fungal siderophores with an important role in medicine, specifically heart and cardiovascular diseases, are coprogen and ferrichrome (both hydroxamate type siderophores). Recent reports described the potential use of these two siderophores produced by fungi as anti-atherosclerotic agents (24, 25). The researchers based their findings on the fact that iron accumulates in atherosclerotic lesions contributing to iron-dependent lipid oxidation. By utilizing siderophores as iron-chelating agents the effects of lipid oxidation can be reduced, representing potential improvement of the disease. Pócsi and co-workers (25) determined that both coprogen (produced by *P. chrysogenum* and *Neurospora crassa*) and ferrichrome (produced by *P. chrysogenum*) reduced the heme-catalyzed low density lipoprotein (LDL) oxidation and its cytotoxic metabolites to human vasculature tissue. The other group studied the stability and antimicrobial and antifungal effects of coprogen produced by *Penicillium nalgiovense* in cheese and sausage sources (24). Other siderophores were also detected in different food sources, including neocoprogen and triacetylfusarinine, and researchers recommended including high-siderophore containing foods in our diets to support protection from cardiovascular diseases.

## Expert opinion

The advantages presented in the literature reviewed here brings to our attention an important issue: there is a need for more studies that will address siderophore production and that will directly connect such a biochemical pathway to the observable physiological patterns to develop relevant application(s). This paradox is observable in bioremediation and plant growth research studies where we lack siderophore structure information and how structure correlates with function and application development. However, this has been addressed to some degree in clinical or medical investigations. The lack in

the correlation of structure with physiological function could hinder the development of significant advances for the fields presented earlier in the review. To develop strong fundamental conclusions and further applications of siderophores, researchers must employ different detection, characterization techniques (liquid chromatography-mass spectrometry, nuclear magnetic resonance or electro spray ionization) and experiments (iron-deficient vs. iron-enriched; genetic manipulations; appropriate controls) to correlate particular molecular characteristics with empirical observations; and principal basis to suggest potential biotechnological applications.

## Outlook

As the interest of siderophore biosynthesis and production from microorganisms is increasing in the scientific community, more studies will cover different approaches for biotechnological applications of siderophores. Accompanied with an increase in scientific knowledge regarding siderophore biotechnology, we will also observe an improvement in the quality of life of those populations affected by disease, environmental contamination, or famine, as biotechnology investigators moves their findings to a ‘bench-to-bedside’ development pipeline. This will bring together researchers from different scientific disciplines with the common objective of improving societies across the world. The efforts in siderophore investigations will uncover the potential of applications in medicine, agriculture and bioremediation, creating a novel industry based on the knowledge obtained. Fostering these studies will establish the commitment of scientists for economic development, stability and continuity in affected societies and communities.

## Highlights

- Iron is an important nutrient for physiological processes in the cell. It is available in two oxidation states: ferric ( $\text{Fe}^{3+}$ ) and ferrous ( $\text{Fe}^{2+}$ )
- Siderophores are small iron-chelating molecules produced at low iron concentrations by microorganisms. These molecules chelate ferric iron
- Siderophore biosynthesis is genetically controlled in an iron concentration-dependent manner. At low-iron concentrations, the Fur protein detaches from the *fur* box, allowing transcription and translation of corresponding siderophore genes

- Agricultural applications of siderophores are mostly based on plant growth, biocontrol and biofertilizer approaches
- In the food industry, siderophores could find a niche as food-grade antioxidants, ensuring food preservation and shelf-life
- Bioremediation applications presented here are mainly based on heavy metal transformations and immobilization due to siderophore-producing microorganisms. Additional examples presented included applications with oil-degrading, siderophore-producing microbes
- Applications of siderophores in medical or pharmacological settings included antimicrobial and conjugate approaches. Additionally, vaccines and radiopharmaceutical imaging options were presented
- There is a need to correlate molecular characteristics of siderophores with their function to translate that in biotechnological applications in medicine, agriculture, food industry, bioremediation and biodegradation of pollutants.

**Acknowledgments:** The author thank the valuable comments and ideas contributed by his former doctoral dissertation co-advisors, Dr. Abigail M. Richards and Dr. Anne K. Camper from the Center for Biofilm Engineering at Montana State University. Many thanks to members of the Center for Translational Medicine at the University of Montana for their support. The author reports no conflict of financial or any other interest that affected the outcome of the article.

## References

1. Sampaio AF, Silva M, Dornas WC, Costa DC, Silva ME, dos Santos RC, de Lima WG, Pedrosa ML. Iron toxicity mediated by oxidative stress enhances tissue damage in an animal model of diabetes. *Biometals* 2014; 27: 349–61.
2. Fernandez-Real JM, Manco M. Effects of iron overload on chronic metabolic diseases. *Lancet Diab Endocrinol* 2014; 2: 513–26.
3. Geetha SJ, Joshi SJ. Engineering rhizobial bioinoculants: a strategy to improve iron nutrition. *Sci World J* 2013; 15: 1–15.
4. Rastogi A, Mishra BK, Singh M, Mishra R, Shukla S. Role of micronutrients on quantitative traits and prospects of its accumulation in linseed (*Linum usitatissimum* L.). *Arch Agron Soil Sci* 2014; 60: 1389–409.
5. Mishra AK, Shukla SK, Yadav DV, Awasthi SK. Iron, manganese and sulphur uptake and nutrients availability in sugarcane based system in subtropical India. *Sugar Tech* 2014; 16: 300–10.
6. Schurmann P. The influence of mineral salt deficiency on chlorophyll formation and the relationship of chlorophyll a and b in green algae. *Ber Schweiz Bot Ges* 1966; 76: 59–96.
7. Machold O. Lamellar proteins of green and chlorotic chloroplasts as affected by iron deficiency and antibiotics. *Biochim Biophys Acta* 1971; 238: 324–31.
8. Crosa JH, Mey AR, Payne SM. Iron transport in bacteria, 1st ed., Washington, DC: ASM Press, 2004: 499.
9. Sandy M, Butler A. Microbial iron acquisition: marine and terrestrial siderophores. *Chem Rev* 2009; 109: 4580–95.
10. Ozaki S, Sato T, Sekine Y, Migita CT, Uchida T, Ishimori K. Spectroscopic studies on HasA from *Yersinia pseudotuberculosis*. *J Inorg Biochem* 2014; 138: 31–8.
11. Krieg S, Hucho F, Diederichs K, Izadi-Pruneyre N, Lecroisey A, Wandersman C, Delepelaire P, Welte W. Heme uptake across the outer membrane as revealed by crystal structures of the receptor-hemophore complex. *Proc Natl Acad Sci USA* 2009; 106: 1045–50.
12. Flannery AR, Renberg RL, Andrews NW. Pathways of iron acquisition and utilization in *Leishmania*. *Curr Opin Microbiol* 2013; 16: 716–21.
13. Tong Y, Guo M. Bacterial heme-transport proteins and their heme-coordination modes. *Arch Biochem Biophys* 2009; 481: 1–15.
14. Patel AK, Deshattiwar MK, Chaudhari BL, Chincholkar SB. Production, purification and chemical characterization of the catecholate siderophore from potent probiotic strains of *Bacillus* spp. *Biores Technol* 2009; 100: 368–73.
15. Ahire JJ, Patil KP, Chaudhari BL, Chincholkar SB. *Bacillus* spp. of human origin: a potential siderophoregenic probiotic bacteria. *Appl Biochem Biotechnol* 2011; 164: 386–400.
16. Kline T, Fromhold M, McKennon TE, Cai S, Treiberg J, Ihle N, Sherman D, Schwan W, Hickey MJ, Warrenner P, Witte PR, Brody LL, Goltry L, Barker LM, Anderson SU, Tanaka SK, Shawar RM, Nguyen LY, Langhorne M, Bigelow A, Embuscado L, Naeemi E. Antimicrobial effects of novel siderophores linked to beta-lactam antibiotics. *Bioorg Medicin Chem* 2000; 8: 73–93.
17. Kobayakawa F, Kodani S. Screening of streptomycetes for production of desferrioxamines. *J Pure Appl Microbiol* 2012; 6: 1553–8.
18. Cohen SM, O'Sullivan B, Raymond KN. Mixed hydroxypyridinonate ligands as iron chelators. *Inorg Chem* 2000; 39: 4339–46.
19. Hordt W, Romheld V, Winkelmann G. Fusarinines and dimeric acid, mono- and dihydroxamate siderophores from *Penicillium chrysogenum*, improve iron utilization by strategy I and strategy II plants. *Biometals* 2000; 13: 37–46.
20. Fu SF, Sun PF, Lu HY, Wei JY, Xiao HS, Fang WT, Cheng BY, Chou JY. Plant growth-promoting traits of yeasts isolated from the phyllosphere and rhizosphere of *Drosera spatulata* Lab. *Fungal Biol* 2016; 120: 433–48.
21. Zhu JY, Zhang JX, Li Q, Han T, Xie JP, Hu YH, Chai L. Phylogenetic analysis of bacterial community composition in sediment contaminated with multiple heavy metals from the Xiangjiang River in China. *Mar Pollut Bull* 2013; 70: 134–9.
22. Farkas G, Gazso LG, Diosi G. Characterization of subterranean bacteria in the Hungarian Upper Permian siltstone (Aleulolite) formation. *Can J Microbiol* 2000; 46: 559–64.
23. Howard DH, Rafie R, Tiwari A, Faull KF. Hydroxamate siderophores of *Histoplasma capsulatum*. *Infect Immun* 2000; 68: 2338–43.
24. Emri T, Toth V, Nagy CT, Nagy G, Pócsi I, Gyemant G, Antal K, Balla J, Balla G, Román G, Kovács I, Pócsi I. Towards high-siderophore-content foods: optimisation of coprogen production in



- submerged cultures of *Penicillium nalgiovense*. J Sci Food and Agricult 2013; 93: 2221–8.
25. Pocsí I, Jeney V, Kertai P, Pocsí I, Emri T, Gyemant G, Fésüs L, Balla J, Balla G. Fungal siderophores function as protective agents of LDL oxidation and are promising anti-atherosclerotic metabolites in functional food. Mol Nutr Food Res 2008; 52: 1434–47.
26. Todokoro T, Fukuda K, Matsumura K, Irie M, Hata Y. Production of the natural iron chelator deferriferrichrysin from *Aspergillus oryzae* and evaluation as a novel food-grade antioxidant. J Sci Food Agricult 2016; 96: 2998–3006.
27. Nair A, Juwarkar AA, Singh SK. Production and characterization of siderophores and its application in arsenic removal from contaminated soil. Water Air Soil Pollut 2007; 180: 199–212.
28. Wolff-Boenisch D, Traina SJ. The effect of desferrioxamine B on the desorption of U(VI) from Georgia kaolinite KGa-1b and its ligand-promoted dissolution at pH 6 and 25 degrees C. Chem Geol 2007; 242: 278–87.
29. Barbeau K, Zhang GP, Live DH, Butler A. Petrobactin, a photoreactive siderophore produced by the oil-degrading marine bacterium *Marinobacter hydrocarbonoclasticus*. J Am Chem Soc 2002; 124: 378–9.
30. Gauglitz JM, Zhou HJ, Butler A. A suite of citrate-derived siderophores from a marine *Vibrio* species isolated following the Deepwater Horizon oil spill. J Inorg Biochem 2012; 107: 90–5.
31. Tomaras AP, Crandon JL, McPherson CJ, Banevicius MA, Finegan SM, Irvine RL, Brown MF, O'Donnell JP, Nicolau DP. Adaptation-based resistance to siderophore-conjugated antibacterial agents by *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2013; 57: 4197–207.
32. Chi Z, Wang XX, Ma ZC, Buzdar MA, Chi ZM. The unique role of siderophore in marine-derived *Aureobasidium pullulans* HN6.2. Biometals 2012; 25: 219–30.
33. Wang WL, Chi ZM, Chi Z, Li J, Wang XH. Siderophore production by the marine-derived *Aureobasidium pullulans* and its antimicrobial activity. Biores Technol 2009; 100: 2639–41.
34. Norman CS. The treatment of iron over load with desferrioxamine B. Irish J Med Sci 1964; 457: 13–8.
35. Shah S, Karkhanis V, Desai A. Isolation and characterization of siderophore, with antimicrobial activity, from *Azospirillum lipoferum* M. Curr Microbiol 1992; 25: 347–51.
36. Edberg F, Kalinowski BE, Holmstrom SJM, Holm K. Mobilization of metals from uranium mine waste: the role of pyoverdines produced by *Pseudomonas fluorescens*. Geobiology 2010; 8: 278–92.
37. Braud A, Jezequel K, Bazot S, Lebeau T. Enhanced phytoextraction of an agricultural Cr- and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere 2009; 74: 280–6.
38. Sagar S, Dwivedi A, Yadav S, Tripathi M, Kaistha SD. Hexavalent chromium reduction and plant growth promotion by *Staphylococcus arlettae* Strain Cr11. Chemosphere 2012; 86: 847–52.
39. Nagata T, Oobo T, Aozasa O. Efficacy of a bacterial siderophore, pyoverdine, to supply iron to *Solanum lycopersicum* plants. J Biosci Bioeng 2013; 115: 686–90.
40. Gadd GM. Influence of microorganisms on the environmental fate of radionuclides. Endeavour 1996; 20: 150–6.
41. Gostincar C, Ohm RA, Kogej T, Sonjak S, Turk M, Zajc J, Zalar P, Grube M, Sun H, Han J, Sharma A, Chiniquy J, Ngan CY, Lipzen A, Barry K, Grigoriev IV, Gunde-Cimerman N. Genome sequencing of four *Aureobasidium pullulans* varieties: biotechnological potential, stress tolerance, and description of new species. BMC Genomics 2014; 15: 1–59.
42. Wenciewicz TA, Long TE, Mollmann U, Miller MJ. Trihydroxamate siderophore-fluoroquinolone conjugates are selective sideromycin antibiotics that target *Staphylococcus aureus*. Bioconj Chem 2013; 24: 473–86.
43. Rout ME, Chrzanowski TH, Westlie TK, DeLuca TH, Callaway RM, Holben WE. Bacterial endophytes enhance competition by invasive plants. Am J Bot 2013; 100: 1726–37.
44. Tani A, Akita M, Murase H, Kimbara K. Culturable bacteria in hydroponic cultures of moss *Racomitrium japonicum* and their potential as biofertilizers for moss production. J Biosci Bioeng 2011; 112: 32–9.
45. Konings AF, Martin LW, Sharples KJ, Roddam LF, Latham R, Reid DW, Lamont IL. *Pseudomonas aeruginosa* uses multiple pathways to acquire iron during chronic infection in cystic fibrosis lungs. Infect Immun 2013; 81: 2697–704.
46. Lee J, Attila C, Cirillo SLG, Cirillo JD, Wood TK. Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. Microb Biotechnol 2009; 2: 75–90.
47. Cornelis P, Matthijs S, Van Oeffelen L. Iron uptake regulation in *Pseudomonas aeruginosa*. Biometals 2009; 22: 15–22.
48. Gull M, Hafeez FY. Characterization of siderophore producing bacterial strain *Pseudomonas fluorescens* Mst 8.2 as plant growth promoting and biocontrol agent in wheat. African J Microbiol Res 2012; 6: 6308–18.
49. Sayyed RZ, Gangurde NS, Patel PR, Joshi SA, Chincholkar SB. Siderophore production by *Alcaligenes faecalis* and its application for growth promotion in *Arachis hypogaea*. Indian J Biotechnol 2010; 9: 302–7.
50. Nabti E, Bensidhoum L, Tabli N, Dahel D, Weiss A, Rothballer M, Schmid M, Hartmann A. Growth stimulation of barley and biocontrol effect on plant pathogenic fungi by a *Cellulosimicrobium* sp. strain isolated from salt-affected rhizosphere soil in northwestern Algeria. Eur J Soil Biol 2014; 61: 20–6.
51. Ravari SB, Heidarzadeh N. Isolation and characterization of rhizosphere auxin producing Bacilli and evaluation of their potency on wheat growth improvement. Arch Agron Soil Sci 2014; 60: 895–905.
52. Yu XM, Ai CX, Xin L, Zhou GF. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. Eur J Soil Biol 2011; 47: 138–45.
53. Woo SM, Kim SD. Structural identification of siderophore(AH18) from *Bacillus subtilis* AH18, a biocontrol agent of *Phytophthora* blight disease in red-pepper. Kor J Microbiol Biotechnol 2008; 36: 326–35.
54. Zhao L, Wang F, Zhao J. Identification and functional characteristics of chlorpyrifos-degrading and plant growth promoting bacterium *Acinetobacter calcoaceticus*. J Basic Microbiol 2014; 54: 457–63.
55. Lin TX, Xu CH, Tang M, Guan QL, Gong MF. Siderophore producing by endophytic bacterial strain YBS106 with antifungal activity against *Fusarium oxysporum*. J Pure Appl Microbiol 2013; 7: 2091–6.
56. Patel AK, Ahire JJ, Pawar SP, Chaudhari BL, Shouche YS, Chincholkar SB. Evaluation of probiotic characteristics of siderophoregenic *Bacillus* spp. isolated from dairy waste. Appl Biochem Biotechnol 2010; 160: 140–55.

57. Gao B, Lu J, Hao H, Yin S, Yu X, Wang Q, Sun K. Heavy metals pollution and Pb isotopic signatures in surface sediments collected from Bohai Bay, North China. *Sci World J* 2014; 2014: 1–6.
58. Baceva K, Stafilov T, Matevski V. Bioaccumulation of heavy metals by endemic viola species from the soil in the vicinity of the As-Sb-Tl mine 'Allchar', Republic of Macedonia. *Int J Phytorem* 2014; 16: 347–65.
59. Gbadebo AM, Ekwue YA. Heavy metal contamination in tailings and rocksamples from an abandoned goldmine in southwestern Nigeria. *Environ Monit Assessmt* 2014; 186: 165–74.
60. De Souza M-J, Bharathi PAL, Nair S, Chandramohan D. 'Trade-off' in Antarctic bacteria: limnetic psychrotrophs concede multiple enzyme expressions for multiple metal resistance. *Biometals* 2007; 20: 821–8.
61. Lin SY, Hameed A, Liu YC, Hsu YH, Lai WA, Chen WM, Shen FT, Young CC. *Pseudomonas sagittaria* sp. nov., a siderophore-producing bacterium isolated from oil-contaminated soil. *Int J System Evol Microbiol* 2013; 63: 2410–7.
62. Matlakowska R, Sklodowska A. The culturable bacteria isolated from organic-rich black shale potentially useful in biometallurgical procedures. *J Appl Microbiol* 2009; 107: 858–66.
63. Chuang CY, Santschi PH, Ho YF, Conte MH, Guo LD, Schumann D, Ayrano M, Li Y-H. Role of biopolymers as major carrier phases of Th, Pa, Pb, Po, and Be radionuclides in settling particles from the Atlantic Ocean. *Mar Chem* 2013; 157: 131–43.
64. Demirkanli DI, Molz FJ, Kaplan DI, Fjeld RA. Soil-root interactions controlling upward plutonium transport in variably saturated soils. *Vadose Zone J* 2009; 8: 574–85.
65. Yasumoto-Hirose M, Nishijima M, Ngirchchol MK, Kanoh K, Shizuri Y, Miki W. Isolation of marine bacteria by *in situ* culture on media-supplemented polyurethane foam. *Mar Biotechnol* 2006; 8: 227–37.
66. Moeschlin S, Schnider U. Treatment of primary and secondary hemochromatosis and acute iron poisoning with a new, potent iron-eliminating agent (Desferrioxamine-B). *N Eng J Med* 1963; 269: 57–66.
67. Goodwin JF, Whitten CF. Chelation of ferrous sulphate solutions by desferrioxamine B. *Nature* 1965; 205: 281–3.
68. Bergeron RJ, Liu ZR, McManis JS, Wiegand J. Structural alterations in desferrioxamine compatible with iron clearance in animals. *J Med Chem* 1992; 35: 4739–44.
69. De la Rosa-Garcia SC, Munoz-Garcia AA, Barahona-Perez LF, Gamboa-Angulo MM. Antimicrobial properties of moderately halotolerant bacteria from cenotes of the Yucatan peninsula. *Lett Appl Microbiol* 2007; 45: 289–94.
70. Silva-Stenico ME, Pamplona Silva CS, Lorenzi AS, Shishido TK, Etchegaray A, Lira SP, Moraes LA, Fiore MF. Non-ribosomal peptides produced by Brazilian cyanobacterial isolates with antimicrobial activity. *Microbiol Res* 2011; 166: 161–75.
71. Minkwitz A, Berg G. Comparison of antifungal activities and 16S ribosomal DNA sequences of clinical and environmental isolates of *Stenotrophomonas maltophilia*. *J Clin Microbiol* 2001; 39: 139–45.
72. Kodani S, Bicz J, Song L, Deeth RJ, Ohnishi-Kameyama M, Yoshida M, Ochi K, Challis GL. Structure and biosynthesis of scabichelin, a novel tris-hydroxamate siderophore produced by the plant pathogen *Streptomyces scabies* 87.22. *Org Biomol Chem* 2013; 11: 4686–94.
73. Ghosh A, Miller MJ. Synthesis of novel citrate-based siderophores and siderophore-beta-lactam conjugates-iron transport-mediated drug-delivery systems. *J Org Chem* 1993; 58: 7652–9.
74. Miller MJ, Walz AJ, Zhu H, Wu CR, Moraski G, Mollmann U, Tristani EM, Crumbliss AL, Ferdig MT, Checkley L, Edwards RL, Boshoff HI. Design, synthesis, and study of a mycobactin-arte-misinin conjugate that has selective and potent activity against tuberculosis and malaria. *J Amer Chem Soc* 2011; 133: 2076–9.
75. Mollmann U, Heinisch L, Bauernfeind A, Kohler T, Ankel-Fuchs D. Siderophores as drug delivery agents: application of the 'Trojan Horse' strategy. *Biometals* 2009; 22: 615–24.
76. Minnick AA, McKee JA, Dolence EK, Miller MJ. Iron transport-mediated antibacterial activity of and development of resistance to hydroxamate and catechol siderophore-carbacephalosporin conjugates. *Antimicrob Agents Chemother* 1992; 36: 840–50.
77. Rivault F, Liebert C, Burger A, Hoegy F, Abdallah MA, Schalk IJ, Mislin GL. Synthesis of pyochelin-norfloxacin conjugates. *Bioorg Med Chem Lett* 2007; 17: 640–4.
78. Souto A, Montaos MA, Balado M, Osorio CR, Rodriguez J, Lemos ML, Jiménez C. Synthesis and antibacterial activity of conjugates between norfloxacin and analogues of the siderophore vanchromycin. *Bioorg Med Chem* 2013; 21: 295–302.
79. Mollmann U, Ghosh A, Dolence EK, Dolence JA, Ghosh M, Miller MJ, Reissbrodt R. Selective growth promotion and growth inhibition of Gram-negative and Gram-positive bacteria by synthetic siderophore-beta-lactam conjugates. *Biometals* 1998; 11: 1–12.
80. Kingsley R, Rabsch W, Williams PH, Reissbrodt R. Iron supply in *Salmonellae* in diagnosis, epidemiology, and in the process of infection. *Immun Infect* 1994; 22: 10–4.
81. Fernandez L, Mendez J, Guijarro JA. Molecular virulence mechanisms of the fish pathogen *Yersinia ruckeri*. *Vet Microbiol* 2007; 125: 1–10.
82. Garenaux A, Caza M, Dozois CM. The Ins and Outs of siderophore mediated iron uptake by extra-intestinal pathogenic *Escherichia coli*. *Vet Microbiol* 2011; 153: 89–98.
83. Mike LA, Smith SN, Sumner CA, Eaton KA, Mobley HLT. Siderophore vaccine conjugates protect against uropathogenic *Escherichia coli* urinary tract infection. *Proc Natl Acad Sci USA* 2016; 113: 13468–73.
84. Sassone-Corsi M, Chairatana P, Zheng T, Perez-Lopez A, Edwards RA, George MD, Nolan EM, Raffatellu M. Siderophore-based immunization strategy to inhibit growth of enteric pathogens. *Proc Natl Acad Sci USA* 2016; 113: 13462–7.
85. Bergeron RJ, Bharti N, Singh S, McManis JS, Wiegand J, Green LG. Vibriobactin antibodies: a vaccine strategy. *J Med Chem* 2009; 52: 3801–13.
86. Brumbaugh AR, Smith SN, Mobley HLT. Immunization with the Yersiniabactin receptor, FyuA, protects against pyelonephritis in a murine model of urinary tract infection. *Infect Immun* 2013; 81: 3309–16.
87. Fox JT, Thomson DU, Drouillard JS, Thornton AB, Burkhardt DT, Emery DA, Nagaraja TG. Efficacy of *Escherichia coli* O157: H7 siderophore receptor/porin proteins-based vaccine in feedlot cattle naturally shedding *E. coli* O157. *Foodborne Path Dis* 2009; 6: 893–9.
88. Mariotti P, Malito E, Biancucci M, Lo Surdo P, Mishra RPN, Nardi-Dei V, Savino S, Nissum M, Spraggon G, Grandi G, Bagnoli F, Bottomley MJ. Structural and functional characterization of the *Staphylococcus aureus* virulence factor and vaccine candidate FluD2. *Biochem J* 2013; 449: 683–93.
89. Petrik M, Franssen GM, Haas H, Laverman P, Hoertnagl C, Schrettl M, Helbok A, Lass-Flörl C, Decristoforo C. Preclinical evaluation of two Ga-68-siderophores as potential radiopharmaceuticals for *Aspergillus fumigatus* infection imaging. *Eur J Nuc Med Molec Imag* 2012; 39: 1175–83.