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# Bacterial siderophores: diversity, uptake pathways and applications

Isabelle J. Schalk

University of Strasbourg, CNRS, Strasbourg, France.

E-mail [schalk@unistra.fr](mailto:schalk@unistra.fr)

## **ABSTRACT**

Iron is an essential nutrient for the growth, survival and virulence of almost all bacteria. To access iron, many bacteria produce siderophores, molecules with a high affinity for iron. Research has highlighted substantial diversity in the chemical structure of siderophores produced by bacteria, as well as remarkable variety in the molecular mechanisms involved in strategies for acquiring iron through these molecules. The metal-chelating properties of siderophores, characterized by their high affinity for iron and ability to chelate numerous other metals (albeit with lower affinity compared to iron), have also generated interest in diverse fields. Siderophores find applications in the environment, such as in bioremediation and agriculture, where emerging and innovative strategies are being developed to address pollution and enhance nutrient availability for plants. Moreover, in medicine, siderophores could be used as tools for novel antimicrobial therapies, medical imaging, as well as in hemochromatosis, thalassemia or cancer treatments. This review offers insights into the diversity of siderophores, highlighting their potential applications in environmental and medical contexts.

1

## 2 [H1] Introduction

3 Iron is a vital nutrient for almost all living organisms, spanning from microorganisms to mammals<sup>1,2</sup>.  
4 Its significance in biology stems from its ability to form bonds with oxygen, the redox reactivity of the  
5  $\text{Fe}^{2+}/\text{Fe}^{3+}$  couple, its catalytic role and its capacity to form metal complexes. As a result, iron has a  
6 crucial role in oxygen transport and functions as an ideal cofactor for enzymes involved in electron  
7 transfer reactions, including those in cellular respiration, DNA synthesis and repair, and antioxidant  
8 defense mechanisms<sup>1,2</sup>. For optimal growth, bacteria require iron concentrations in the range of  $10^{-5}$   
9 to  $10^{-7}$  M Ref.<sup>3</sup>. However, these conditions are seldom met in aerobic environments because iron is  
10 poorly bioavailable. In the presence of oxygen and at neutral pH, iron primarily exists as ferric iron  
11 (Fe(III)), which is highly insoluble and precipitates as various insoluble iron oxide compounds (solubility  
12 equilibrium constant:  $K_s(\text{Fe}(\text{OH})_3) = 2,8 \times 10^{-39}$  for  $\text{Fe}^{3+}$  versus  $K_s(\text{Fe}(\text{OH})_2) = 4,9 \times 10^{-17}$  for  $\text{Fe}^{2+}$ ). In the  
13 context of host infections, iron is also scarcely bioavailable, as the host actively limits the availability  
14 of this metal to pathogens through a strategy known as nutritional immunity<sup>4-10</sup>. This strategy involves  
15 proteins such as ferritin (an iron storage protein) that sequester iron. Additionally, the host produces  
16 hormones and proteins that regulate iron availability ; for example, the hormone hepcidin is produced  
17 in response to inflammation and reduces iron absorption in the intestine, thereby limiting iron  
18 availability for bacteria. Finally, iron-binding proteins such as lactoferrin, transferrin and calprotectin  
19 are produced at the infection site, rendering it unavailable. This array of strategies leads to a reduced  
20 concentration of free ferric iron in human body fluids and the infection sites.

21 A prevalent strategy bacteria use to access iron relies on siderophores, which are renowned for their  
22 exceptionally high affinity for ferric iron<sup>11,12</sup> (Table 1). Siderophores are secondary metabolites that  
23 are synthesized by microorganisms through biosynthetic pathways involving several enzymes,  
24 including non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS)<sup>13,14</sup>. Generally,  
25 they exhibit molecular weights ranging from 200 to 2000 Da. Once synthesized, siderophores are  
26 excreted into the bacterial environment to scavenge iron and transport it back into the bacteria  
27 through specialized transporters<sup>15</sup>. Afterwards, iron release from siderophores in the bacterial cells  
28 occurs via various molecular mechanisms<sup>16</sup>. Often, microorganisms can produce 2-3 different  
29 siderophores, and they possess the ability to exploit siderophores that have been produced by other  
30 microorganisms (xenosiderophores)<sup>17</sup>.

31 In addition to chelating iron, siderophores can also chelate numerous other metals, including biological  
32 metals like Cu, Co, Ni, toxic metals like Al, Ga, Pb, or radionuclides like U, albeit with lower affinity  
33 compared to iron<sup>18,19</sup>. Due to their metal-chelating properties, siderophores have increasingly found

1 applications in recent years as biofertilizers, biopesticides, and agents for controlling pathogens in  
2 agriculture and aquaculture<sup>20–23</sup>. In bioremediation, they chelate toxic metals, aiding environmental  
3 cleanup<sup>24</sup>. Siderophores also show promise in iron chelation therapy, antibiotic delivery and medical  
4 imaging<sup>25,26</sup>. In cancer research, they may hinder cancer cell growth<sup>27,28</sup>.

5 In this Review, I highlight and discuss recent advances in our understanding of the diverse molecular  
6 mechanisms involved in siderophore-mediated iron acquisition in bacteria. We now recognize that  
7 these mechanisms can be exceptionally varied and more complex than those previously described for  
8 *Escherichia coli*. This diversity of molecular mechanisms is exemplified by the pathogen *Pseudomonas*  
9 *aeruginosa*, which exhibits numerous iron import pathways that are mediated by siderophores<sup>12</sup>.  
10 Furthermore, as the range of applications for siderophores continues to expand across various fields,  
11 including agriculture and medicine, the technical potential of these bacterial secondary metabolites  
12 are also discussed.

13

## 14 **[H1] Iron acquisition using siderophores**

15 In general, siderophore-mediated iron acquisition by bacteria involves a series of steps: sensing iron  
16 starvation; synthesizing and secreting siderophores; chelating iron; importing the siderophore-Fe  
17 complexes via specific transport systems; and finally releasing iron from these siderophores, inside the  
18 bacterial cells.

19 The transcriptional repressor Fur (ferric uptake regulator) is the major actor to sense iron starvation.  
20 As long as iron levels are sufficient, Fur binds ferrous iron (Fe<sup>2+</sup>) as a cofactor, undergoing a  
21 conformational change that enables it to bind to specific DNA sequences called Fur boxes in the  
22 promoter regions of target genes encoding proteins like those involved in siderophore biosynthesis  
23 and iron acquisition by siderophores<sup>29–31</sup>. Under conditions of iron limitation, intracellular iron levels  
24 decrease, which leads to the dissociation of iron from Fur. Consequently, Fur undergoes a structural  
25 change that reduces its DNA-binding affinity, which results in the de-repression of the target genes  
26 transcription<sup>32</sup>. In addition to Fur regulation, other layers of control exist at the post-transcriptional  
27 and translational regulation levels mediated by riboswitches and small RNAs<sup>33,34</sup>. All these mechanisms  
28 ensure that the bacterium can efficiently acquire iron while responding to changes in iron availability.  
29 For more details, see the following reviews and articles<sup>31,35–42</sup>.

30

## 31 **[H2] The biosynthesis of siderophores and its cellular organization.**

1 Out of the 500 different siderophores identified, only a handful have had their biosynthetic pathways  
2 thoroughly investigated and elucidated. Enterobactin, which is produced by *E. coli*, was the first  
3 siderophore for which the biosynthesis pathways has been reported<sup>43</sup>. Since then, the biosynthesis of  
4 pyoverdine I and pyochelin, which are produced by *P. aeruginosa*<sup>14</sup>, mycobactins<sup>44</sup>, which is produced  
5 by *Mycobacterium tuberculosis*, and yersiniabactin<sup>13,45,46</sup>, which is produced by *Yersinia pestis*, have  
6 also been described. The biosynthesis of siderophores invariably entails a multi-step process that is  
7 coordinated by specific enzymes. It commences with the synthesis of precursor molecules, typically  
8 amino acids, aromatic rings and small carboxylic acids, which are subsequently assembled to form the  
9 final siderophore structure by NRPS or PKS<sup>44,47-49</sup>. Further refinement of the nascent siderophore  
10 structure may occur through acylation and cyclization. The mature siderophore molecule is then  
11 released into the extracellular environment. NRPS assemble peptides by linking amino acids together,  
12 whereas PKS construct molecules by joining together acetyl, malonyl or methylmalonyl units, typically  
13 forming polyketides. Both NRPS and PKS are composed of several modules, with each module being  
14 responsible for adding a specific building block, enabling the synthesis of diverse and structurally  
15 complex molecules (see the following reviews for more details on NRPS and PKS<sup>50-52</sup>).

16 The first-described biosynthesis of a siderophore was that of enterobactin, a tris-catecholate  
17 siderophore that binds ferric iron through three 2,3-dihydroxybenzoate (DHB) groups. DHB is  
18 synthesized from chorismate by three enzymes: EntA, EntB and EntC (with EntC functioning as an  
19 isochorismate synthase, EntB as an isochorismatase, and EntA as a 2,3-dihydro-2,3-dihydroxybenzoate  
20 dehydrogenase)<sup>46,53,54</sup> (Figure 1). The subsequent stage involves the assembly of three molecules of  
21 DHB and three L-serines to form a molecule of enterobactin. This assembly is facilitated by the NRPS  
22 EntF and three additional enzymes: EntE, EntB and EntD<sup>55</sup>. DHB is activated by EntE and transferred to  
23 EntB. The NRPS EntF catalyzes the formation of each ester and amide bond during the assembly of the  
24 three DHB units. EntD functions as a 4'-phosphopantetheinyl transferase, necessary for activating one  
25 of the sub-domains of EntF and EntB.

26 Enterobactin biosynthesis involves one NRPS (EntF), but most siderophore biosynthesis pathways  
27 involve multiple NRPS or PKS enzymes, as well as complex maturation processes of the synthesized  
28 molecule. This complexity is evident in the biosynthesis pathways of pyoverdine I. Pyoverdines  
29 constitute a family of chromo-peptide siderophores produced by fluorescent *Pseudomonas* strains,  
30 with pyoverdine I, II, and III representing distinct variants within this family, each distinguished by its  
31 unique structure<sup>56,57</sup>. Pyoverdine I functions as the primary siderophore produced by *P. aeruginosa*  
32 PAO1, whereas pyoverdine II and III are alternative variants produced by other strains of *P. aeruginosa*.  
33 The core structure of pyoverdine I comprises an eleven-amino-acid peptide that is assembled by four  
34 NRPSs (PvdL, PvdI, PvdJ, and PvdD)<sup>14,58</sup> (Figure 2). Within this peptide backbone, two unusual amino

1 acids, L-2,4-diaminobutyrate (L-Dab) and L-N5-formyl-N5-hydroxyornithine (L-fOHOrn), are  
2 incorporated, with L-Dab being synthesized by the aminotransferase PvdH<sup>59</sup> and L-fOHOrn being  
3 generated by the L-ornithine-N5-oxygenase PvdA and the hydroxyornithine transformylase PvdF<sup>60-62</sup>.  
4 Additionally, during the initial assembly by NRPSs, a myristic or myristoleic acid is likely to be linked to  
5 the first amino acid of the peptide to ensure the association of the pyoverdine precursor with the  
6 bacterial inner membrane during synthesis<sup>14,58</sup>. After its assembly by the NRPSs, the pyoverdine  
7 precursor is translocated from the cytoplasm into the periplasm by the inner membrane ATP-binding-  
8 cassette (ABC) transporter PvdE<sup>63</sup>. In the periplasm, the myristic or myristoleic acid moiety is removed  
9 by the acylase PvdQ<sup>64,65</sup>. Moreover, a portion of the peptide undergoes an auto-cyclization (involving  
10 L-Dab and D-Tyr residues) to form the characteristic chromophore of pyoverdines. This cyclization  
11 process requires the copper-dependent tyrosinase PvdP<sup>66,67</sup> and the oxidoreductase PvdO<sup>68,69</sup>. Finally,  
12 the release of siderophores into the bacterial environment involves efflux systems, with PvdRT–OpmQ  
13 being responsible for the secretion of pyoverdine I (Refs. <sup>70,71</sup>).

14

15 Proteomic analyses of isolated inner membranes of *P. aeruginosa* have provided evidence that a  
16 portion of each of the four NRPSs involved in pyoverdine I biosynthesis (PvdL, PvdI, PvdJ, and PvdD) is  
17 associated with the inner membranes and forms protein complexes<sup>72</sup>. This enzymatic complex was  
18 initially termed a ‘siderosome’ in the literature. However, since the term siderosome also refers to a  
19 membrane-bound organelle found in spleen macrophages, containing aggregates of hemosiderin, we  
20 will avoid using the term siderosome in this review to prevent any confusion. Instead, we will use the  
21 term multi-enzymatic complex.

22 More recently, multicolor single-molecule localization microscopy approaches showed colocalization  
23 between PvdL and the other three NRPS<sup>73</sup>, which further suggests the existence of a multi-enzymatic  
24 complex and that PvdL may have a central role in the assembly of this complex at the level of the inner  
25 leaflet of the cytoplasmic membrane. Concerning the other cytoplasmic enzymes involved in  
26 pyoverdine I biosynthesis, pull-down assays using PvdA, the enzyme responsible for producing L-  
27 fOHOrn in *P. aeruginosa*, have revealed its interaction with NRPS PvdJ and PvdL<sup>72</sup>. Co-purification  
28 assays have also demonstrated that PvdA binds to PvdF, with both enzymes catalyzing fOHOrn  
29 synthesis<sup>74</sup>. Furthermore, in vivo single-molecule tracking and Förster resonance energy transfer, as  
30 measured by fluorescence lifetime imaging, demonstrated that PvdA predominantly diffuses while  
31 bound to large complexes in the cytoplasm of *P. aeruginosa*, with a small, exchangeable trapped  
32 fraction<sup>75,76</sup>. PvdA was also found to interact with different stoichiometries with the four NRPS (PvdL,  
33 PvdI, PvdJ, and PvdD): several PvdA molecules interacted with PvdI, whereas PvdA formed one-to-one  
34 complexes with PvdJ<sup>76</sup>. All of these data provide further confirmation of the existence of multi-

1 enzymatic complexes associated with the inner membrane and composed of all four NRPSs, PvdA and  
2 PvdF (concerning PvdH no data are currently available) (Figure 2C).

3 Currently, it remains unknown how the association of these multi-enzymatic complexes involved in  
4 pyoverdine I biosynthesis occurs with the inner membrane. PvdA possesses a hydrophobic, inner-  
5 membrane-anchoring domain at its N-terminus, facilitating its association with the inner  
6 membrane<sup>72,77</sup>. PvdA could help PvdL association with the inner membrane. Furthermore, the  
7 presence of a myristic acid chain attached to the first amino acid of the pyoverdine I backbone has also  
8 led to the suggestion that pyoverdine I biosynthesis takes place at the inner leaflet of the cytoplasmic  
9 membrane<sup>65</sup>. The association of PvdL and possibly all multi-enzymatic complexes involved in  
10 pyoverdine I biosynthesis to the cytoplasmic membrane may occur via PvdA, the myristic acid chain of  
11 the pyoverdine I precursor, or through some other unknown mechanism.

12 This discovery of multi-enzymatic complexes for the biosynthesis of pyoverdine I offers a completely  
13 novel perspective on the organization of siderophore biosynthesis in bacterial cells<sup>72,75,76,78,79</sup>. Such  
14 organizations probably also exist for the synthesis of other siderophores in other bacterial species. A  
15 similar spatial organization may also exist for the NRPS involved in the biosynthesis of pyochelin, with  
16 the NRPS PchE co-localizing with the enzyme PchA<sup>78</sup>. Moreover, the fatty acid coenzyme-A ligase  
17 FadD1 has been demonstrated to be necessary for optimal pyochelin production in *P. aeruginosa*  
18 cells<sup>80</sup>, which suggests that in the case of pyochelin biosynthesis a siderophore precursor also exists  
19 with a fatty acid chain. For mycobactin in *Mycobacterium tuberculosis*, the fatty acid ligase FadD33  
20 (also known as MbtM) has been reported to transfer a lipophilic aliphatic chain to the  $\epsilon$ -amino group  
21 of the lysine mycobactin core, thus having a role in the biosynthesis of this siderophore<sup>81</sup>. Moreover,  
22 EntD, one of the enzymes in *E. coli* involved in enterobactin biosynthesis, has been identified as a  
23 membrane protein: membrane fractionation techniques and protease accessibility studies have  
24 indicated that certain domains of EntD are associated with the inner leaflet of the cytoplasmic  
25 membrane<sup>82</sup>. These various observations have led to the hypothesis that siderophore biosynthesis may  
26 occur in proximity to the cytoplasmic membranes.

27 The spatial co-localization of enzymes within a multi-enzymatic complex located by the inner  
28 membrane represents a significant advantage. This organization optimizes the efficient transfer of  
29 siderophore precursors among different enzymes and prevents the dispersion of these precursors  
30 throughout the cytoplasm, where they could potentially chelate intracellular metals. Such organization  
31 may also be important for the secretion of the synthesized siderophores out of the bacterial cells.  
32 Many questions remain regarding the organization of enzymes in such a multi-enzymatic complex.  
33 How do NRPSs interact with each other within the complex? What impact does this organizational

1 structure have on the enzymatic activity of each individual enzyme? Are the enzymes within the  
2 complex continually active in the production of siderophores?

3

## 4 **[H2] Iron chelation by siderophores and high diversity in their chemical** 5 **structures.**

6 Siderophores possess a remarkable ability to scavenge and capture even trace amounts of iron from  
7 their surroundings due to their high affinity for iron ions, especially ferric iron ( $\text{Fe}^{3+}$ ) (Table 1). This  
8 heightened affinity can be attributed to their chemical structure and the presence of bidentate  
9 constituents such as catecholates, hydroxamates,  $\alpha$ -hydroxy-carboxylates and phenolate groups<sup>12,83</sup>  
10 (Figure 3). Siderophores often consist of three bidentate groups, which in an octahedral geometry  
11 ~~around the~~ coordinate with  $\text{Fe}^{3+}$  ions<sup>12,83,84</sup>. However, it is not uncommon to find siderophores with  
12 fewer than three bidentate groups, in which case, the coordination stoichiometry may be 2:1 or 3:1  
13 for siderophore: $\text{Fe}^{3+}$ .

14 Currently, approximately 500 siderophores with diverse chemical structures have been identified,  
15 which highlights the vast variability in their chemical compositions. As mentioned above, whereas  
16 some siderophores exclusively contain catecholate, hydroxamate or  $\alpha$ -hydroxy-carboxylate chelating  
17 groups, many others consist of mixtures of different ligands<sup>12,83,85</sup>. This structural heterogeneity  
18 reflects the adaptive strategies used by microorganisms in diverse ecological niches to thrive under  
19 varying iron availability conditions. These intricate variations in their structures arise from the diverse  
20 enzymatic pathways involved in their biosynthesis across different microbial species.

21 Although siderophores are primarily known for their high affinity for iron, they also coordinate many  
22 other metal ions, albeit with lower affinities compared to iron<sup>18,19</sup> (see below). For example, the  
23 formation constants for the hydroxamate siderophore desferrioxamine B with  $\text{Ga}^{3+}$ ,  $\text{Al}^{3+}$ , and  $\text{In}^{3+}$  range  
24 between  $10^{20}$  and  $10^{28} \text{ M}^{-1}$ , whereas that with  $\text{Fe}^{3+}$  is  $10^{30} \text{ M}^{-1}$  (Refs. <sup>18,86</sup>). Similarly, formation constants  
25 for pyoverdine with  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Mn}^{2+}$  fall between  $10^{17}$  and  $10^{22} \text{ M}^{-1}$ , whereas that with  $\text{Fe}^{3+}$  is  $10^{32}$   
26  $\text{M}^{-1}$  (Ref. <sup>87</sup>). Screening with sixteen different metals ( $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Eu}^{3+}$ ,  $\text{Ga}^{3+}$ ,  $\text{Hg}^{2+}$ ,  
27  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Tb}^{3+}$ ,  $\text{Tl}^+$  and  $\text{Zn}^{2+}$ ) revealed that pyoverdine and pyochelin, the two siderophores  
28 produced by *P. aeruginosa*, are capable of chelating all these metals<sup>88,89</sup>. Another example is with  
29 vanadium, which can be chelated by the siderophores pyochelin, putrebactin, pyoverdine,  
30 enterobactin, and desferrioxamine B<sup>90-93</sup>.

31



## 1 [H2] Uptake and delivery of iron by siderophores.

2 In Gram-negative bacteria, all reported cases have demonstrated that, regardless of the type of  
3 siderophore or bacterial species, iron uptake through siderophores begins with the uptake of  
4 siderophore–Fe complexes across the outer membrane by TonB-dependent transporters (TBDTs)<sup>15</sup>.  
5 These transporters are named after the inner membrane protein TonB, which forms a complex with  
6 two other inner membrane proteins, ExbB and ExbD. The TonB–ExbB–ExbD complex functions as a  
7 molecular ‘motor’, that transmits energy from the inner membrane proton motive force to the TBDT  
8 in the outer membrane, facilitating the translocation of the siderophore-iron complex into the  
9 periplasm<sup>94–96</sup>. These TBDTs demonstrate remarkable specificity in binding to siderophores<sup>15</sup>.  
10 Consequently, when a bacterial species can use different siderophores to access iron, it will express  
11 distinct TBDTs, each tailored to a specific siderophore or closely related ones<sup>15,17,97,98</sup> (Figure 4a). For  
12 instance, *P. aeruginosa* can express at least 14 different TBDTs involved in siderophore-mediated iron  
13 acquisition via siderophores or xenosiderophores<sup>17</sup> (Supplementary Table 1). Furthermore, *E. coli*  
14 strains use up to nine TBDTs, for iron uptake by siderophores or xenosiderophores, and *Salmonella*  
15 species use up to six TBDTs, all dedicated to the utilization of siderophores or xenosiderophores<sup>97–99</sup>  
16 (Supplementary Table 1). It is important to note that even among strains of a species, there can be  
17 strain-to-strain variability in the number of siderophores produced, xenosiderophores used, and genes  
18 encoding TBDTs present in the genomes. Moreover, the presence of various iron uptake pathways in  
19 bacterial genomes underscores the significance of iron for bacterial survival and indicates the existence  
20 of intense competition among microorganisms for this essential resource.

21

22 In Gram-positive bacteria, no outer membrane is present, and the bacterial cells feature specific  
23 receptors anchored in the cytoplasmic membrane that recognize and bind siderophore–iron  
24 complexes. Upon binding, these proteins transfer the siderophore–iron complex to an ABC transporter  
25 embedded in the cytoplasmic membrane<sup>100</sup>. Like TBDTs in Gram-negative bacteria, these ABC  
26 transporters, along with their corresponding membrane-bound receptors, exhibit high siderophore  
27 specificity. For example, *Staphylococcus aureus* can express multiple systems at its cell surface, each  
28 dedicated to importing iron via a specific type of siderophore or xenosiderophore<sup>100,101</sup> (Figure 4c).

29 In both, Gram-negative and Gram positive bacteria, the subsequent critical step in iron acquisition  
30 involves releasing iron from the siderophore–iron complex. In all the mechanisms reported to date,  
31 iron reduction occurs. In Gram-negative bacteria, this step can occur either in the periplasm or  
32 cytoplasm, depending on the siderophore pathway or bacterial species<sup>16</sup> (Figure 4B). In cases of  
33 cytoplasmic dissociation, siderophore–Fe complexes must traverse the inner membrane, where ABC

1 transporters primarily participate in this step, though some protonmotive-dependent transporters  
2 have also been documented<sup>16</sup>. Dissociating ferric iron from high-affinity siderophores necessitates  
3 specific mechanisms, which can vary among siderophores and bacterial species. These strategies  
4 invariably entail iron reduction, often accompanied by enzymatic modification or cleavage of the  
5 siderophore<sup>16</sup>. Additionally, changes in pH can influence the stability of the siderophore–iron complex,  
6 with lower intracellular pH weakening the siderophore–iron binding. A vivid illustration of this  
7 molecular diversity can be found in the case of enterobactin (Figure 5). In *E. coli*, enterobactin  
8 undergoes enzymatic hydrolysis by the esterase Fes in the bacterial cytoplasm, which leads to the  
9 release of the iron for intracellular utilization<sup>85,102</sup> (Figure 5a). By contrast, in *P. aeruginosa*, which uses  
10 enterobactin without producing it, the esterase PfeE hydrolyzes enterobactin in the bacterial  
11 periplasm<sup>103</sup> (Figure 5a). In both cases, iron reduction is also necessary to achieve efficient iron release  
12 from enterobactin<sup>103,104,104</sup>. Another example highlighting this diversity is the iron uptake by  
13 pyoverdine I (Figure 5b), the primary siderophore used by *P. aeruginosa*. Here, iron is released from  
14 the chelator in the bacterial periplasm through a mechanism involving iron reduction, with no chemical  
15 modification of the siderophore<sup>105–107</sup>. Once iron is liberated from the siderophore in the periplasm, it  
16 must traverse the inner membrane to access the cytoplasm for utilization. Gram-negative bacteria use  
17 specialized ABC transporters to facilitate this crucial step<sup>108</sup>.

18 Whether in Gram-negative or Gram-positive bacteria, various reductases involved in the process of  
19 dissociating iron from siderophores have been described (for a recent review, see Ref. <sup>109</sup>). Some of  
20 these reductases are soluble, whereas others are membrane-anchored. Among them are siderophore-  
21 interacting proteins (SIPs), which are flavoproteins. One notable example is the YqjH protein from *E.*  
22 *coli*, which reduces iron in enterobactin–iron and dicitrate–iron complexes<sup>85</sup>. Additionally, a few  
23 reductases have been identified that utilize a [2Fe-2S] cluster, known as the ferric siderophore  
24 reductase (FSR) family<sup>110</sup>. These reductases are found in Gram-positive bacteria like in *Bacillus*  
25 *halodurans* and in Gram-negative bacteria, exemplified by the FhuF protein, implicated in iron release  
26 mechanisms from hydroxamate-type siderophores in *E. coli*<sup>110–112</sup>. Furthermore, a recent discovery  
27 introduces a new class of siderophore–Fe membrane reductases, including FpvG and FoxB, involved in  
28 iron acquisition by pyoverdine and desferrioxamine G in *P. aeruginosa*<sup>107,113</sup>. The X-ray structure of  
29 FpvG reveals a fold with two tightly bound heme molecules that likely have a role in the iron reduction  
30 process<sup>113</sup>. These mechanisms of iron dissociation from siderophores remain poorly understood and  
31 have received much less scientific attention compared to other aspects of iron import by siderophores.

32

33 In conclusion, siderophores exhibit remarkable diversity in their chemical structures (Figure 2),  
34 reflecting probably their adaptation to diverse ecological niches and iron-scavenging strategies. One  
35 can imagine that this structural diversity has arisen through evolutionary processes, with bacteria

1 developing siderophores featuring specific chemical attributes optimized for efficient iron scavenging  
2 in varying environments, contributing to their ecological success and survival. The evolution of  
3 siderophore chemical structures has likely occurred alongside a diversification of mechanisms for  
4 dissociating siderophore-Fe complexes in bacteria, often displaying distinct and complex mechanisms.  
5 These mechanisms of iron release from siderophores can also vary between species for the same  
6 siderophore as shown with the example of iron acquisition by enterobactin in *E. coli* and *P. aeruginosa*.  
7

## 8 **[H2] Uptake and delivery of other metals than iron by siderophores.**

9 Siderophores can chelate many metals besides iron<sup>89,114</sup>, but whether these metals are imported into  
10 bacteria by siderophores remains a frequently asked question. Since an iron reduction step is most  
11 often essential for releasing iron from the siderophore and delivering it to bacterial cells, one could  
12 assume that no metal other than iron would be delivered to bacteria by siderophores. This iron  
13 reduction step, necessary for releasing iron from siderophores, may be a critical control point for the  
14 metal selectivity of these iron uptake pathways, helping to avoid the release of metals other than iron  
15 from siderophores. In *P. aeruginosa*, it has been shown that pyoverdine–metal complexes other than  
16 pyoverdine–Fe are re-excreted by efflux systems because probably the metal cannot be removed from  
17 the siderophore<sup>115</sup>.

18 However, data also show the opposite — that siderophores can import other biologically relevant  
19 metals besides iron. It was shown that yersiniabactin can bind several divalent transition metals,  
20 including Ni and Cu, and that *E. coli* can use yersiniabactin to acquire Cu and Ni during metal  
21 limitation<sup>116</sup>. Moreover, it has been shown that the probiotic bacterium *E. coli* Nissle 1917 (or ‘Nissle’)  
22 utilizes yersiniabactin to import Zn, enabling Nissle to grow in zinc-limited media<sup>117</sup>. Additionally,  
23 yersiniabactin in *Yersinia pestis* has been shown to overcome zinc limitation during infection of both  
24 mammalian and insect hosts<sup>118</sup>. All these data suggest that yersiniabactin behaves more like a  
25 metallophore than a siderophore. However, this behavior may be specific to yersiniabactin or  
26 siderophores with chemical structures similar to yersiniabactin. Pyochelin, like yersiniabactin, contains  
27 a phenolate–thiazoline core in its structure (Figure 3) and is capable of transporting metals other than  
28 iron into *P. aeruginosa* cells<sup>88</sup>. However, it has not been confirmed whether these uptakes have  
29 biological significance. Currently, the mechanism of iron or metal release from yersiniabactin or  
30 pyochelin is unknown, and it may involve siderophore degradation rather than iron reduction. It would  
31 be interesting to investigate whether siderophores with chemical structures similar to yersiniabactin  
32 can also facilitate the uptake of other biologically relevant metals into bacteria, potentially having a  
33 crucial role in bacterial physiology and virulence.  
34

## 1 [H1] Siderophores in competition and infection

2 Within bacterial communities, a fierce competition unfolds among microorganisms for resources, and  
3 siderophores have a pivotal role in this competition for iron. Studies have demonstrated that reduced  
4 iron availability leads to the proliferation of siderophore-producing bacterial families and decreases in  
5 the abundance of non-siderophore iron-dependent taxa within the intestinal microbiota, underscoring  
6 the significance of siderophores<sup>119,120</sup>. Furthermore, microbial species within a microbiome that  
7 produce siderophores generate a variety of these molecules with distinct chemical structures, which  
8 results in varying affinities for iron. Additionally, bacteria often use their own siderophores to access  
9 iron and can also exploit siderophores produced by other bacteria (xenosiderophores) by expressing  
10 the necessary transporters (TBDT in Gram-negative bacteria or ABC transporters in Gram-positive  
11 bacteria, as depicted in Figure 4). As a result, the diverse microorganisms within a microbiome exhibit  
12 differential capabilities to acquire iron, which substantially affects their growth and survival within the  
13 microbiota<sup>121</sup>. Some bacteria may outcompete others by producing or utilizing siderophores with  
14 higher iron-binding affinities, thereby gaining a competitive edge in iron-limited environments. For  
15 example, bacteria with the ability to use a wide range of siderophores, such as *P. aeruginosa*  
16 (Supplementary table 1), are more competitive than *Salmonella enterica* subsp. *enterica* serovar  
17 Typhimurium within bacterial communities. This competition for iron mediated by siderophores can  
18 influence the composition and dynamics of the entire microbial community<sup>121</sup>. Moreover, siderophore  
19 production by beneficial bacteria may contribute to pathogen exclusion in the microbiota<sup>121</sup>.

20 Although the full extent of the importance of siderophores in microbial communities and iron  
21 competition remains incompletely understood, key factors in this competition undoubtedly include  
22 the affinity of siderophores for iron and the ensuing competition for the chelation of this metal; and  
23 the ability of the bacteria to use an extensive array of siderophores, particularly those with high iron  
24 affinity.

25 During infection, a battle for iron occurs between the host and pathogens. As mentioned in the  
26 introduction, the host uses a defense strategy known as nutritional immunity, whereby essential  
27 nutrients such as iron are restricted from pathogens<sup>4-9</sup>. To overcome this iron restriction, bacteria  
28 produce and release siderophores, which scavenge iron from the host, including from proteins like  
29 transferrin and ferritin<sup>122,123</sup>. By acquiring iron, bacteria can enhance their growth and survival within  
30 the host, thereby promoting the establishment and progression of infection<sup>124</sup>. Additionally, pathogens  
31 can exploit xenosiderophores produced by other microorganisms, including commensal or competing  
32 ones, further complicating the interplay between pathogens, the host immune system and the  
33 microbiota<sup>10,125</sup>. Due to their crucial role in infection, siderophores are often regarded as critical  
34 virulence factors that contribute to the pathogenesis of bacterial infections by facilitating iron

1 acquisition and promoting bacterial survival and proliferation within the host environment.  
2 Understanding the role of siderophores in infection can offer insights into novel therapeutic strategies  
3 targeting bacterial iron acquisition pathways.

4

## 5 **[H1] Versatile tools for innovative applications**

6 Due to their high affinity for iron and their ability to chelate metals other than iron with strong  
7 affinities, siderophores have emerged as innovative, potent, and versatile tools in various fields,  
8 including agriculture, bioremediation, metal recovery and medical applications (Table 2). Below, I have  
9 briefly outlined these various applications to provide an overview of the potential uses for these  
10 molecules.

11

### 12 **[H2] Application in agriculture.**

13 Siderophores hold promise for enhancing agricultural practices, particularly as biofertilizers and  
14 biopesticides, offering benefits such as improved plant growth, nutrient uptake and overall crop  
15 health<sup>126</sup>.

16 As previously discussed for bacteria, iron is also an essential nutrient for plant growth. However, iron  
17 often exists in insoluble forms in soils. Even though plants produce their own siderophores to access  
18 iron (phytosiderophores), the additional presence of siderophore-producing bacteria can aid in  
19 solubilizing iron by solubilizing it from various insoluble iron oxide compounds (Supplementary Figure  
20 1a), thus making it more accessible for plant absorption, thus preventing iron deficiency chlorosis (for  
21 a review see Ref. <sup>127</sup>). Additionally, as siderophores can also form complexes with other biological metal  
22 ions, such as Zn, Cu and Mn, the presence of siderophore-producing bacteria in the soil may enhance  
23 the solubility and availability of these nutrients to plants<sup>127</sup>. This leads to improved nutrient uptake  
24 and enhanced plant growth.

25 Furthermore, siderophores can have a role in protecting plants against certain pathogens by disrupting  
26 their iron acquisition processes (Supplementary Figure 1b). This contribution leads to improved plant  
27 health and reduced disease incidence. For instance, *Bacillus* spp. inhibits the growth of *Fusarium*  
28 *oxysporum* by producing siderophores that scavenge iron from the environment<sup>128,129</sup>. These findings  
29 underscore how the use of siderophores or siderophore-producing microorganisms can contribute to  
30 more sustainable and efficient agricultural practices, ultimately resulting in increased crop productivity  
31 and improved soil health (reducing the need for synthetic chemical fertilizers).

1 Siderophores have also demonstrated potential in the biocontrol of fish pathogens in aquaculture  
2 settings<sup>130–133</sup>. Aquatic farming, including fish, shellfish and aquatic plants, often faces challenges  
3 related to disease outbreaks caused by various pathogens. Beneficial microorganisms that produce  
4 siderophores can outcompete fish pathogens for available iron in the environment. This competition  
5 reduces the growth and survival of the pathogens, aiding in the control of disease outbreaks. The use  
6 of siderophores as biocontrol agents can help reduce the reliance on chemical treatments for  
7 managing fish diseases, thereby minimizing the risk of antibiotic resistance.

8 It is essential to note that although the potential benefits of using siderophores in agriculture and  
9 aquaculture are promising, practical implementation necessitates careful consideration of factors such  
10 as specific pathogens, the microbial community within agricultural or aquaculture environments, and  
11 potential effects on non-target organisms.

12

## 13 **[H2] Application in bioremediation.**

14 Siderophores have also displayed potential for applications in bioremediation, a process that uses  
15 living organisms or their products to detoxify or remove pollutants from contaminated environments  
16 (for a review see Ref. <sup>24</sup>). As already mentioned above, siderophores possess the ability to chelate a  
17 wide range of metals, including toxic ones such as heavy metals (Ar, Cd, Pb, Hg, Cr, Ni, Cu, etc.),  
18 metalloids (Te or Po) and radionuclides (U), although their affinities for these metals are generally  
19 lower compared to their affinity for Fe<sup>3+</sup> (Ref. <sup>134</sup>). Consequently, siderophores can have a substantial  
20 role in remediating environments that are contaminated with metal pollutants. When released into  
21 contaminated environments, siderophores can bind to these toxic metal ions, forming stable  
22 complexes that increase the solubility and availability of metals for uptake by microorganisms through  
23 iron uptake pathways. For instance, a study identified siderophore production by *Pseudomonas*  
24 *brenneri* MF957286 as a tool for removing Cr(VI) from contaminated soils<sup>135</sup>. Marine bacteria-  
25 produced siderophores have been found to chelate polonium (Po) and protactinium (Pa), whereas  
26 pyoverdine produced by *P. fluorescence* has demonstrated its ability to chelate uranium (Ur)<sup>136,137</sup>.  
27 Another research group increased siderophore-mediated bioaccumulation of Cd(II) by *Bacillus*  
28 *subtilis*<sup>138</sup>. Additionally, it was shown that *P. azotoformans* produces a mixed-type catecholate and  
29 hydroxamate siderophore when grown on arsenic-contaminated soil, which was more efficient than  
30 citric acid or EDTA in removing arsenic from the soil<sup>139</sup>. However, it is important to note that  
31 complexation with siderophores does not guarantee the entry and accumulation of toxic metals or  
32 metalloids within bacterial cells. When bioaccumulation of siderophore–metal complexes occurs, it  
33 can often be toxic to bacteria, affecting their growth. In response, bacteria may develop various

1 resistance mechanisms, such as increasing the expression of efflux systems<sup>140</sup>. To overcome such  
2 challenges, physical methods like precipitation or biosorption can be used to remove siderophore-  
3 metal complexes from contaminated soils, liquids or waste<sup>141</sup>.

4 Siderophores can also be integrated with other bioremediation techniques, including microbial  
5 consortia and phytoremediation (Supplementary Figure 1c), to create synergistic effects and enhance  
6 overall remediation outcomes<sup>142</sup>. In phytoremediation, a bioremediation strategy that use plants to  
7 extract pollutants from soil, siderophores can improve metal solubilization and subsequent metal  
8 uptake by plants (Supplementary Figure 1c). A study using siderophore-producing *P. aeruginosa*, *P.*  
9 *fluorescens* and *Ralstonia metallidurans* showed that rhizobial inoculation with these species in maize  
10 plants increased the phytoremediation of chromium (Cr) and lead (Pb) <sup>143</sup>.

11 Finally, studies have also indicated that siderophores can mitigate the toxicity of asbestos, silicate  
12 minerals present in soil that have been used in construction for many years. Asbestos is highly toxic  
13 due to its substantial iron content, which generates free radicals and reactive oxygen species (ROS)  
14 through the Fenton reaction, which can lead to DNA damage. Inhalation of asbestos fibers can result  
15 in severe health issues, including lung fibrogenesis, mesothelioma, pleural calcification and various  
16 cancers. Iron can be extracted from asbestos fibers using siderophores or siderophore-producing  
17 bacteria (for a review see Ref. <sup>144</sup>).

18 In summary, siderophores hold great promise as tools for enhancing bioremediation efforts within a  
19 range of bioremediation strategies.

20

## 21 **[H2] Recovering rare earth elements.**

22 The ability of siderophores to efficiently chelate metals beyond iron<sup>134</sup> make them valuable candidates  
23 in processes for the recovery of rare earth elements (for a review see Ref. <sup>145</sup>). These elements  
24 encompass lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm),  
25 samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium  
26 (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), scandium (Sc) and yttrium (Y). Rare earth elements  
27 have pivotal roles in various modern technologies, including electronics, renewable energy and  
28 advanced materials. The recovery of these elements from diverse sources holds significant interest  
29 due to their strategic importance and limited global availability. Siderophores could be instrumental  
30 in developing methods for selectively recovering specific rare earth elements from complex mixtures.  
31 They are capable of forming stable complexes with rare earth elements, rendering them more soluble  
32 in aqueous solutions<sup>145,146</sup>. This complexation process facilitates the extraction and separation of rare

1 earth elements from other metals in various solutions, presenting a more environmentally friendly  
2 alternative to traditional extraction methods that involve harsh chemicals.

3

#### 4 **[H2] Metal sensors.**

5 The high affinity of siderophores for metal ions has proven valuable in the development of metal  
6 sensors or methods for metal detection (for a review see Ref. <sup>26,147</sup>). Siderophore-based metal sensors  
7 find application in monitoring metal contamination in environmental samples, including water, soil  
8 and sediment, offering insights into metal pollution levels in ecosystems. Furthermore, siderophores  
9 can be used to detect metal ions in biological samples. In the field of medical diagnostics, siderophores  
10 have been explored as tools for detecting abnormal metal ion concentrations in bodily fluids, which  
11 could indicate certain health conditions<sup>148,149</sup>.

12 In certain cases, the spectral properties of siderophores have been directly used as visual indicators of  
13 the presence of metal ions. Some siderophores undergo spectral changes, including variations in  
14 fluorescence and/or absorbance, upon binding to specific metal ions<sup>89,150,151</sup>. These alterations in  
15 fluorescence or absorbance can be detected and quantified, which enables the sensitive detection of  
16 metal ions. Siderophores can be modified and covalently linked to fluorophores; when such conjugates  
17 bind to a metal ion, the fluorescence properties of the probe change<sup>148,149</sup>. Siderophore-modified  
18 electrodes, known as electrochemical sensors, can be used<sup>152</sup>. The binding of metal ions to the  
19 siderophore on the electrode surface can induce changes in electrical properties, which can be  
20 measured and correlated with the concentration of the metal ion. Siderophores can also be  
21 incorporated into nanoparticle structures to create sensitive metal sensors, referred to as  
22 nanoparticle-based sensors<sup>153,154</sup>. Furthermore, siderophores can be combined with genetically  
23 engineered bacteria or other microorganisms to develop bioreporters<sup>155</sup>. These bioreporters emit  
24 fluorescence or other signals when they encounter specific metal ions, providing a live, biological  
25 indicator of metal presence. These sensors offer advantages such as specificity, sensitivity, and  
26 potential adaptability for on-site or real-time detection.

27

#### 28 **[H2] Imaging agent.**

29 Siderophores can be modified to carry imaging agents, such as radioactive isotopes or contrast agents  
30 used in various imaging modalities (for example, positron emission tomography, single-photon  
31 emission computed tomography, magnetic resonance imaging (MRI))<sup>156</sup>. For example, siderophores  
32 can enhance the contrast in MRI images by binding to certain metal ions, which affects the relaxation



1 properties of nearby water molecules. This can improve the visibility of specific tissues or structures  
2 during MRI scans<sup>157,158</sup>. These modified siderophores can be designed to selectively accumulate in  
3 specific tissues or cells of interest (Supplementary Figure 1e). Such imaging agents can detect bacterial  
4 infections in small animal imaging experiments<sup>159</sup>. Modified siderophores can be engineered to  
5 target cancer cells that overexpress specific receptors. For example, pyoverdine was conjugated to  
6 synthesized superparamagnetic iron oxide nanoparticles. These superparamagnetic iron oxide  
7 nanoparticles-pyoverdine conjugates were covalently bound to a MUC1 aptamer<sup>160</sup>. An  
8 aptamer is a short, single-stranded oligonucleotide that can bind to a specific target molecule  
9 with high affinity and specificity. The conjugate could be used as a diagnostic agent, since a  
10 type of MUC1 is aberrantly overexpressed on the majority of cancer cell surfaces, such as in  
11 human colon cancer. The superparamagnetic iron oxide nanoparticles-pyoverdine-MUC1  
12 complex was also loaded with doxorubicin, an anti-cancer agent, to create a targeted drug  
13 delivery system<sup>160</sup>. This compound was detected at the tumor site in mice using ex vivo  
14 fluorescence imaging. In vivo analysis revealed significant tumor inhibition and increased  
15 survival rates in the mice receiving this compound compared to untreated mice. Inflammation is  
16 often associated with increased iron metabolism, and siderophores labeled with imaging agents can  
17 be used to detect and monitor inflammatory processes in various conditions<sup>161</sup>. The use of  
18 siderophores in imaging is an area of ongoing research and innovation<sup>162-165</sup>.

19

## 20 **[H2] Iron chelation therapy.**

21 Iron chelation therapy is a medical treatment aimed at reducing excess iron levels in the body, which  
22 can occur due to conditions such as hereditary hemochromatosis, thalassemia and certain types of  
23 anemias<sup>166</sup>. In this approach, synthetic siderophores or siderophore analogs can be used to bind excess  
24 iron in the body, and the resulting iron complexes are excreted through urine and feces<sup>28,167</sup>. The  
25 remarkably affinity of siderophores for iron ions enables them to efficiently and selectively bind to  
26 excess iron in the bloodstream and tissues, making this affinity crucial for the success of iron chelation  
27 therapy. Desferrioxamine (Desferal), deferasirox (Exjade), and deferiprone (Ferriprox) (Supplementary  
28 Figure 1f) have been the most important US FDA-approved iron chelators over the past years<sup>28,167</sup>. DFO  
29 is a natural siderophore, whereas the other two compounds are synthetic molecules.

30

## 31 **[H2] Cancer treatment.**

1 Siderophores have also garnered attention in the context of cancer research. Cancer cells often require  
2 higher levels of iron to fuel their rapid growth and proliferation. In fact, a link exists between iron  
3 overload and the progression of cancer (for a review see Ref. <sup>168</sup>). Consequently, cancer cells frequently  
4 manifest alterations in iron metabolism and heightened expression of iron-regulating proteins.  
5 Researchers have explored the idea of using siderophores or siderophore-like compounds to  
6 specifically address the heightened iron requirements of cancer cells. The fundamental concept behind  
7 this approach is to hinder the growth of cancer cells by depriving them of the iron essential for their  
8 survival. For example, ferrichrome has demonstrated a robust capacity to suppress colon cancer cells  
9 without impeding the growth of non-cancerous intestinal cells<sup>169</sup>. Similarly, desferrioxamine has  
10 undergone testing against metastatic hepatocellular carcinoma<sup>170</sup>. However, such an approach is  
11 complex and necessitates thorough consideration of potential side effects and interactions with  
12 normal cells. To address this challenge, numerous artificial iron chelators, including pyridoxal  
13 isonicotinoyl hydrazone, thiosemicarbazones and O-tresox, have been developed for cancer therapy  
14 (for a review see Ref. <sup>28,171</sup>). However, currently no compound has received approval for clinical use.

15

## 16 **[H2] Pathogen sensor.**

17 Due to the strong, specific, and selective interaction between siderophore–Fe complexes and their  
18 transporters, such as TBDTs in the case of Gram-negative bacteria<sup>15</sup>, siderophores have been  
19 considered in the development of pathogen sensors. These sensors utilize the interaction between  
20 siderophores and pathogens to create detection methods that are capable of identifying the presence  
21 of harmful microorganisms (for reviews see Refs. <sup>26,147</sup>). Siderophores are modified with a fluorescent  
22 or colorimetric tag. When the siderophore–Fe complex binds to a specific pathogen (binding to the  
23 TBDT at the bacterial surface), fluorescence or color changes occur, indicating the presence of the  
24 pathogen (Supplementary Figure 1e). Another strategy involves using siderophores in conjunction with  
25 bioreporter systems, where the presence of the pathogen induces a specific response in a genetically  
26 engineered organism. This response can be easily detectable, providing a visible indicator of pathogen  
27 presence. For instance, researchers have developed a sensor system based on siderophores and gold-  
28 plated chips, combined with microscopy analysis, that is capable of detecting and distinguishing  
29 *Yersinia enterocolitica*, *S. aureus*, *Mycobacterium smegmatis* and *P. aeruginosa*<sup>172</sup>. Others immobilized  
30 pyoverdine on planar aluminum chip substrates and used Raman microspectroscopy to isolate and  
31 identify *P. aeruginosa* and *P. fluorescence* from tap water<sup>173</sup>. These different sensors offer rapid  
32 detection, as the interaction between the siderophore and pathogen can occur relatively quickly in

1 various environments, including food, water, clinical samples and clinical diagnostics, producing a  
2 visible or measurable signal.

3

## 4 **[H2] Vectorization of antibiotics.**

5 One of the bottlenecks in the development of new antibiotics is the ability of these drugs to enter  
6 bacteria and especially to cross the outer membrane of Gram-negative bacteria. Iron-uptake systems  
7 that use siderophores can function as pathways for drug import into bacteria. Indeed, by covalently  
8 attaching antibiotics to siderophores, these compounds can exploit the bacterial iron uptake pathway  
9 to deliver antibiotics directly into bacterial cells<sup>174</sup> (Supplementary Figure 1d). This strategy was not  
10 invented by scientists, but rather, bacteria have evolved such compounds to outcompete and kill other  
11 bacteria for resources. Natural siderophore-antibiotic conjugates, such as albomycins, ferrimycins,  
12 danomycins or salmycins, are produced by microorganisms<sup>175</sup>. As each bacterial species uses its own  
13 siderophores, and not necessarily those of other bacterial species, it is possible to design siderophore-  
14 conjugated antibiotics to target specific bacterial species or strains. Many different siderophore-  
15 antibiotics have been designed and tested<sup>176–178</sup>. The results have shown promising in vitro activity  
16 against various clinically relevant multidrug-resistant Gram-negative bacteria. However, these  
17 compounds have not yet made it to the market, except for Cefiderocol<sup>179–181</sup>. Cefiderocol was  
18 developed by Shionogi and approved by the USA Food and Drug Administration in 2019 and European  
19 Medicines Agency in 2020, respectively, and was released on the market of the United States of  
20 America and Europe. Cefiderocol contains a cephalosporin moiety linked to a catechol functional  
21 group, which acts as an iron chelator (for a review see Ref. <sup>182</sup>). The uptake of this compound occurs  
22 through the iron-uptake pathways and in the periplasm, it inhibits the bacterial cell wall synthesis,  
23 ultimately leading to cell death. As with any antibiotic, the development of resistance is inevitable.  
24 Regarding the resistance mechanisms observed so far against cefiderocol, the most frequently  
25 reported mechanisms are those observed against  $\beta$ -lactams, as well as reduced expression or mutation  
26 of genes encoding the TBDTs involved in cefiderocol-iron uptake (for a review, see Ref. <sup>182</sup>). The  
27 example of cefiderocol demonstrates that iron import pathways can be promising import pathways  
28 for antibiotics when vectorized by siderophores. However, resistance phenomena cannot be avoided,  
29 and the development of this type of strategy requires a thorough understanding of the biology related  
30 to the expression and functioning of these iron import pathways in bacteria, particularly in pathogenic  
31 bacteria.

32

33

## 1 [H1] Conclusion

2 In conclusion, the diversity of siderophore chemical structures and the array of molecular mechanisms  
3 involved in iron acquisition by siderophores reflect the intricate interplay between microorganisms  
4 and their environments, and underscores the remarkable adaptability of microorganisms to acquire  
5 this essential nutrient in various environments. These molecular mechanisms, which encompass  
6 siderophore biosynthesis, iron chelation, and iron import and delivery into bacterial cells, enable  
7 microorganisms to thrive in iron-limited ecosystems and contribute to the dynamics of microbial  
8 communities. Understanding the complexity of siderophore-mediated iron acquisition is crucial for  
9 elucidating microbial physiology, ecology and evolution, as well as for developing strategies to use  
10 siderophores and the siderophore-dependent microbial iron uptake pathways for environmental,  
11 biotechnological and biomedical applications. Indeed, siderophores, with their diverse chemical  
12 structures and exceptional metal-chelating properties, represent fascinating and versatile molecules  
13 offering opportunities for innovative applications across multiple disciplines, and they may unlock  
14 innovative solutions for diverse challenges.

15

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## 15 **Competing interest.**

16 The author declares no competing interests.

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21 Supplementary information is available for this paper at <https://doi.org/10.1038/s415XX-XXX-XXXX-X>

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1 **Table 1: Stability constants of siderophores for iron.**

Siderophore	Chelating groups	Denticity	Stability constant ( $K_a$ )	REFERENCE
Enterobactin	Catechols	6	$10^{49} \text{ M}^{-1}$	183
Protochelin	Catechols	6	$10^{44} \text{ M}^{-1}$	184
Pyoverdine	Catechol or Hydroxamates	6	$10^{32} \text{ M}^{-1}$	57
Nocardamine	Hydroxamates	6	$10^{32} \text{ M}^{-1}$	185
Desferrioxamine B	Hydroxamates	6	$10^{32} \text{ M}^{-1}$	186
Desferrichrome	Hydroxamates	6	$10^{29} \text{ M}^{-1}$	185
Pyochelin	Hydroxyphenylthiazolone	4	$10^{18} \text{ M}^{-2}$	187
Rhizoferrin	Carboxylic acid	4	$10^{25} \text{ M}^{-1}$	188

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**Table 2: Applications of siderophore properties in various fields, ranging from agriculture to bioremediation and medical science.**

Field of applications	Specific utilization of the siderophore	References
<b>AGRICULTRE</b>		
Biofertilizers	Improve nutrient (iron and other biological metals) uptake and enhance plant growth	127
Biopesticides	Protect plants against certain pathogens by disrupting their iron acquisition processes	128,129
Aquaculture settings	Biocontrol of fish pathogens by disrupting their iron acquisition processes	130–133
<b>DEPOLLUTION</b>		
Bioremediation	Ability to chelate and remove heavy metals, metalloids and radionuclides from contaminated wastes or environments	24
Recovering rare earth elements	Recovery of rare earth elements cobalt (Co), nickel (Ni), lanthanum (La), praseodymium (Pr), neodymium (Nd), lithium (Li), dysprosium (Dy)	145
Metal sensor	Monitoring metal contamination in environmental samples and in ecosystems	26,147
<b>BIOMEDICAL</b>		
Metal sensor	Detection of abnormal metal ion concentrations in bodily fluids (medical diagnostics)	148,149
Imaging agent	Siderophores can be modified to carry imaging agents, such as radioactive isotopes or contrast agents	162–165
Iron chelation therapy	Reduce excess iron levels in the body of patient with hemochromatosis, thalassemia, and certain types of anemias	166
Anticancer	Hinder the growth of cancer cells by depriving them of the iron essential for their survival	28,171
Pathogen sensor	Strategy based on to the strong, specific, and selective interaction between siderophore-Fe complexes and their transporters, such as TBDT in the case of Gram-negative bacteria)	26,147
Antibiotics vectorization		176–178

	Iron uptake systems utilizing siderophores can serve as pathways for drug import into bacteria	
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**Figure 1. Biosynthesis pathway of enterobactin in *Escherichia coli*.** The biosynthesis of enterobactin involves the synthesis of 2,3-dihydroxybenzoate (DHB) from chorismate by three enzymes: EntA, EntB and EntC. Enterobactin is then formed by assembling three molecules of DHB and three L-serines, facilitated by the non-ribosomal peptide synthetases (NRPS) EntF together with EntE and EntD enzymes. EntF catalyzes the formation of ester and amide bonds, whereas EntD functions as a 4'-phosphopantetheinyl transferase that is essential for activating sub-domains of EntF and EntB. The stereochemistry of the chemical structures of the siderophore presented is not specified.

**Figure 2. Biosynthesis pathway of pyoverdine I in *Pseudomonas aeruginosa*.** a) The precursor of the siderophore pyoverdine I is an 11-amino acid peptide that is assembled in the cytoplasm of *Pseudomonas aeruginosa* cells by four non-ribosomal peptide synthetases (NRPS), namely PvdL, PvdI, PvdJ and PvdD. Additionally, three enzymes (PvdH, PvdA and PvdF) are involved in synthesizing atypical amino acids utilized by the NRPS. PvdL is the NRPS involved in the assembling of the tripeptide L-Glu–L-Tyr–D-Dab (in blue like PvdL). Next, L-Ser, L-Arg, L-Ser and L-OHOrn (all in green) are incorporated successively by PvdI, L-Lys and L-OHOrn (in pink) by PvdJ and the two L-Thr (in yellow) by PvdD. From the initial step of peptide assembly, a myristate or myristoleate chain is incorporated on the first residue (L-Glu) by PvdL. b) The cytoplasmic precursor is exported to the periplasm, where it undergoes maturation involving other enzymes (PvdQ, PvdP and PvdO), which leads to the removal of the fatty acid chain and cyclization of the chromophore. c) **The relative size of the enzymes is shown.** PvdL, PvdI, PvdD and PvdD are NRPS, with very different molecular weights (480, 570, 240 and 274 kDa for PvdL, PvdI, PvdJ and PvdD respectively). The molecular weights of three enzymes, PvdH, PvdA and PvdF, involved in synthesizing atypical amino acids are 50, 50 and 31 kDa. d) **Schematic view of the multi-enzymatic complex formed between the different enzymes involved in pyoverdine 1 biosynthesis in *P. aeruginosa*.** The gene organization is illustrated, along with a schematic view of the multi-enzymatic complex formed by PvdL–PvdI–PvdJ–PvdD–PvdH–PvdF–PvdA (colours as in part c). Parts c and d is adapted with permission from Gasser et al., 2020 (Ref. <sup>75</sup>).

**Figure 3. Structures of siderophores.** Shown are structures of different siderophores. Highlighted are in blue the catechol chelating groups; in green the hydroxamates; in pink the carboxylates; and in orange the phenolates. The stereochemistry of the chemical structures of the siderophores presented is not specified.

1 **Figure 4. Iron uptake in Gram negative and Gram positive bacteria. a) Siderophore-Fe transport**  
2 **through the outer membrane in Gram-negative bacteria.** Iron uptake begins with the translocation of  
3 siderophore-Fe complexes across the outer membrane by TonB-dependent transporters (TBDTs)<sup>15</sup>.  
4 Each siderophore or closely related siderophore has a specific TBDT that facilitates its uptake by the  
5 bacterial cells. The energy required for this transport is provided by the TonB–ExbB–ExbD complex.  
6 This complex functions as a molecular ‘motor’, that transmits energy from the inner membrane proton  
7 motive force to the TBDT in the outer membrane, facilitating the translocation of the siderophore-iron  
8 complex into the periplasm<sup>94–96</sup>. More TBDTs are expressed than TonB–ExbB–ExbD complexes,  
9 indicating that a single TonB–ExbB–ExbD complex interacts with several TBDTs. I modified the text to  
10 better explain the message of Figure 4a.] **b) Iron release from siderophores** In Gram-negative  
11 bacteria. After the uptake of siderophore-Fe complexes across the outer membrane, the release of  
12 iron from the siderophore can occur either in the periplasm or the cytoplasm, depending on the  
13 siderophore and bacterial species<sup>16</sup>. In the first case, after its dissociation from the chelator, iron  
14 associates with periplasmic binding proteins (PBP) to avoid any precipitation and is then transported  
15 across the inner membrane by a specific ABC transporter. In the second case, the siderophore-Fe binds  
16 in the periplasm to a PBP associated with an ABC transporter, which transports the siderophore-Fe  
17 complex further across the inner membrane. Iron release from siderophores always involves an iron  
18 reduction step, which can be associated or not with a chemical modification or hydrolysis of the  
19 siderophore. **c) Iron acquisition by siderophores in Gram-positive bacteria, exemplified by iron**  
20 **uptake in *Staphylococcus aureus*<sup>100,101</sup>.** *S. aureus* produces two hydroxy acid-type siderophores,  
21 staphyloferrin A and staphyloferrin B, along with a hydroxamate-type siderophore containing citric  
22 acid residues called staphylobactin. It can also utilize hydroxamate (such as ferrichrome,  
23 desferrioxamine B, aerobactin and coprogen) and catecholate-type xenosiderophores. Another  
24 siderophore possibly produced by *S. aureus* is aureochelin, mentioned in a single article from 1997  
25 (Ref. <sup>189</sup>), with no recent literature to support the finding or identification of the transporter involved.  
26 Hence, aureochelin is not included in the figure. Although its structure remains unknown, it might  
27 resemble a phenolate catecholate-type siderophore. Iron uptake by staphyloferrin A and B,  
28 staphylobactin, and xenosiderophores involves specific ABC transporters associated with receptors  
29 anchored in the inner membrane. Upon entry into the cytoplasm, iron is released from the  
30 siderophores through mechanisms involving iron reduction and pathway-specific mechanisms.  
31 Although the Fhu system has been extensively characterized in *S. aureus*, homologs have been  
32 identified in *L. monocytogenes*<sup>190</sup>, the *B. cereus* group<sup>191</sup>, and *S. pyogenes*<sup>192</sup>. Like *S. aureus*, species  
33 within the *Staphylococcus*, *Listeria*, and *Bacillus* genera can utilize exogenous catecholate siderophores  
34 via the Sst uptake system, and these bacterial species can also import iron through this uptake pathway  
35 using catecholamine stress hormones such as epinephrine, norepinephrine and dopamine<sup>191,193–198</sup>.

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**Figure 5. Iron acquisition by enterobactin and pyoverdine I. a) Iron acquisition by enterobactin in *P.***

***aeruginosa* and *E. coli*.** Iron acquisition by enterobactin (Ent) in *P. aeruginosa* and *E. coli* is a process characterized by distinct mechanisms in each bacterium. *P. aeruginosa* does not produce the enterobactin siderophore but can utilize it. Upon the formation of the Ent–Fe complex in the environment of *P. aeruginosa*, recognition is facilitated by the TonB-dependent transporters (TBDTs) PfeA and PirA, with PfeA acting as the primary TBDT for this siderophore–Fe complex and PirA as the secondary TBDT<sup>199–201</sup>. Following transport across the outer membrane by TBDTs, the iron complex dissociates from Ent in the periplasm. This step involves iron reduction and hydrolysis of Ent into three molecules of 2,3-dihydroxybenzoic acid (DHBS) catalyzed by the esterase PfeE<sup>103</sup>. In contrast, in *E. coli*, a single TBDT, FepA, is involved in the import of the Ent–Fe complex across the outer membrane<sup>202–204</sup>. Within the periplasm, the Ent–Fe complex binds to the periplasmic protein FepB, which is associated with an ATP-binding cassette transporter, FepBC, facilitating import across the inner membrane<sup>205–207</sup>. The dissociation of iron from Ent in the cytoplasm involves a reductase, Ydjh, and an esterase, Fes, similar to the process observed in the periplasm of *P. aeruginosa*, resulting in the hydrolysis of Ent into three DHBS molecules<sup>104,104</sup>.

**b) Iron acquisition by pyoverdine I in *P. aeruginosa*.** Pyoverdine I (Pvd) [Au: pyoverdine I throughout? Yes] is the primary siderophore synthesized and excreted by *P. aeruginosa*. When Pvd has chelated iron in the bacterial environment, the complex is recognized and transported across the outer membrane by two TBDTs, namely FpvA and FpvB<sup>208–210</sup>. FpvA is the major transporter, whereas FpvB functions as the secondary transporter<sup>211,212</sup>. Within the periplasm, the Pvd–Fe complex associates with two periplasmic proteins, FpvF and FpvC<sup>108</sup>. FpvF belongs to the periplasmic siderophore binding protein, and FpvC to the periplasmic iron binding proteins<sup>108,213</sup>. The FpvF–Pvd–Fe–FpvC complex further associates with the inner membrane complex FpvGHJK, where FpvG functions as a reductase, and the functions of the other three proteins (FpvH, FpvJ and FpvK) remain unknown but are essential for optimal reductase activity of FpvG<sup>106,107,214</sup>. Iron is reduced within the FpvCFGHJK complex and dissociated from Pvd<sup>106,107</sup>. The ferrous iron is then specifically chelated by FpvC<sup>213</sup>, which transports it to the ABC transporter FpvDE for translocation into the cytoplasm<sup>108</sup>. Pvd remains associated with FpvF, which guides it to the PvdRT–OpmQ efflux pump, facilitating its export into the extracellular milieu<sup>214–216</sup>. As Pvd undergoes no chemical modification or degradation during the iron release process, it can readily chelate another ferric ion, initiating the cycle anew<sup>105</sup>.

**Table of content:**

In this Review, Schalk explores the molecular mechanisms involved in siderophore-mediated iron acquisition in bacteria. In addition, the possible applications for siderophores in the environment, agriculture and medicine are also discussed.