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REVIEW

Pseudomonas aeruginosa and its multiple strategies to access iron

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Email: schalk@unistra.fr**Abstract**

Pseudomonas aeruginosa is a ubiquitous bacterium found in many natural and man-made environments. It is also a pathogen for plants, animals, and humans. As for almost all living organisms, iron is an essential nutrient for the growth of *P. aeruginosa*. The bacterium has evolved complex systems to access iron and maintain its homeostasis to survive in diverse natural and dynamic host environments. To access ferric iron, *P. aeruginosa* is able to produce two siderophores (pyoverdine and pyochelin), as well as use a variety of siderophores produced by other bacteria (mycobactins, enterobactin, ferrioxamine, ferrichrome, vibriobactin, aerobactin, rhizobactin and schizokinen). Furthermore, it can also use citrate, in addition to catecholamine neuromediators and plant-derived mono catechols, as siderophores. The *P. aeruginosa* genome also encodes three heme-uptake pathways (heme being an iron source) and one ferrous iron acquisition pathway. This review aims to summarize current knowledge concerning the molecular mechanisms involved in all the iron and heme acquisition strategies used by *P. aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous gram-negative bacterium that was first isolated in 1884 by Carle Gessard, a military pharmacist and pastor. It is found in soil, water, and most man-made environments worldwide. It can live in both normal and oxygen-poor environments. *P. aeruginosa* is a pathogen for plants, animals, and humans. It is almost harmless for healthy individuals, but dangerous for those with reduced immunity. The opportunistic pathogenicity of *P. aeruginosa*, mainly in a nosocomial context, was not recognized until the second half of the 20th century. Currently, *P. aeruginosa* is classified as one of the major agents responsible for nosocomial infections, accounting for approximately 10%–15% of hospital-acquired infections. This high prevalence is explained by the metabolic versatility of *P. aeruginosa*, which allows its ubiquitous existence, and its high intrinsic

antibiotic resistance. It is also a major cause of morbidity and mortality for people with cystic fibrosis.

P. aeruginosa, as almost all living organisms, needs iron to grow. Iron is an essential nutrient for most living organisms, in particular, because of its ability to facilitate electron transfer by changing its oxidation state, ranging from elemental iron (0) up to iron VI (Sánchez et al., 2017). These oxidation states are all useful in biology and, among other functions, this metal is involved in aerobic respiration (ferredoxin, cytochromes), DNA synthesis and repair (through its role as a cofactor for helicases, nucleases, glycosylases, and demethylases), and the metabolism of cytochrome-dependent xenobiotics (Dev & Babitt, 2017). Paradoxically, despite the major role played by iron in many biological processes, it is poorly bio-available under aerobic conditions, forming insoluble iron hydroxides(III) at biological pH. Only iron(II), present in anaerobic conditions, is soluble in aqueous medium. Ferrous iron is also an element of

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significant toxicity: iron(II) catalyzes the Fenton reaction ($\text{Fe}^{2+}(\text{aq}) + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+}(\text{aq}) + \text{OH}^-(\text{aq}) + \text{HO}^\bullet$), resulting in the release of reactive oxygenated species (ROS) capable of damaging biomolecules, such as DNA. Iron is the most abundant metal in the human body, but because of its insolubility and toxicity, it is not free, but bound to metalloproteins or present as heme. During an infection, *P. aeruginosa* competes with the host for iron and has developed several strategies to steal it. It is able to acquire iron by importing heme, ferrous iron, or the poorly soluble ferric iron using siderophores (low molecular weight iron chelators). This pathogen is able to produce two siderophores, pyoverdine and pyochelin, as well as use many siderophores produced by other bacteria (exosiderophores) in a piracy strategy. Exosiderophores used by *P. aeruginosa* include mycobactins, enterobactin, ferrioxamin, ferrichrome, vibriobactin, aerobactin, rhizobactin, schizokinen, and even citrate. Recently it has also been demonstrated that mono catechols, such as catecholamine neuromediators and plant-derived catechols, can be used as siderophores by *P. aeruginosa* (Luscher et al., 2022; Perraud et al., 2022). The uptake of both heme and ferric iron via siderophores involves specific transporters found in the outer membrane of all Gram-negative bacteria, called TonB-dependent transporters (TBDTs). In this review, we will first quickly summarize what is a siderophore and the characteristics of TBDTs. We then describe the current knowledge of the various iron- and heme-uptake pathways identified in *P. aeruginosa* in detail at the molecular level.

SIDEROPHORES

P. aeruginosa, as most bacteria, produces and uses siderophores to overcome the problem of iron bioavailability. Siderophores (meaning «iron carriers» in Greek) are small organic molecules (molecular weight between 200 and 2000 Da) that are distinguished by their strong affinity for ferric iron. These molecules are synthesized by bacteria via enzymes and secreted into their environment to acquire iron. The first siderophore to be isolated was ferrichrome A from the fungus *Ustilago sphaerogena* by John Brian Neilands in 1952 (Neilands, 1952). The biological role of siderophores in the acquisition of ferric iron was identified by Garibaldi and Neilands in 1956 (Garibaldi & Neilands, 1956). Since then, more than 500 siderophores with very diverse chemical structures have been identified (Hider & Kong, 2011). As ferric iron is a relatively hard Lewis acid, its preferred ligands are hard Lewis bases, such as deprotonated alcohols. Consequently, the major chelating functions in siderophores are hydroxamates (ex: ferrichrome), alpha-hydroxycarboxylates (ex: rhizoferrin) or alpha-aminocarboxylates (ex: stap hyloferrins), catecholates (ex: enterobactin), hydrox

yphenyloxazolines (ex: mycobactins), and hydroxyphenylthiazolines (ex: pyochelin) (Figure 1). The coordination geometry of ferric iron is octahedral, thus involving six covalent coordination bonds, which can be provided by one, two, or three siderophore molecules, depending on the denticity of the siderophore (Boukhalfa & Crumbliss, 2002; Hider, 1984). The stoichiometry of the ferric complex, as well as the nature of the ligands present on the siderophore, in general, determine its affinity for iron. The affinity of a small number of siderophores (relative to their diversity) for iron has been determined. In general, the determination of pFe ($-\log[\text{Fe}^{3+}]$, by analogy with pH) in the presence of a siderophore at a pH of 7.4 is a relevant estimate of the iron affinity of a siderophore under biological conditions (Albrecht-Gary et al., 1994; Albrecht-Gary & Crumbliss, 1998; Brandel et al., 2012).

TONB-DEPENDENT TRANSPORTERS (TBDT)

TBDTs in general

Siderophores with chelated iron or heme molecules are recognized on the surface of Gram-negative bacteria and imported into the periplasm by highly selective outer membrane transporters called TonB-dependent transporters (TBDTs) (for a review, see Schalk et al., 2012). This name is related to the fact that the uptake of ferric-siderophores by these transporters is energy-dependent and exploits the proton gradient between the periplasm and cytoplasm via molecular machinery called the TonB complex and composed of three inner membrane proteins, TonB, ExbB, and ExbD (Celia et al., 2016, 2019, 2020).

TBDTs are part of a highly conserved family of proteins that possess C-terminal transmembrane β -barrels composed of 22 anti-parallel β -strands, with large disordered extracellular loops connecting the β -strands and short periplasmic loops (Figure 2) (Brillet et al., 2007, 2011; Moynié et al., 2017, 2019). The pore of the barrel is closed by the N-terminus, forming a globular domain called «plug» (Figure 2). The interaction of ferric siderophores with TBDTs first involves recognition by a binding site located in the extracellular loops. The bound ferric siderophore is then recognized by a second site composed of residues of the plug and β -barrel domains (Buchanan et al., 1999; Josts et al., 2019; Moynié et al., 2019; Payne et al., 1997). This second interaction allows the conformational changes in the TBDTs necessary for the import of ferric-siderophores. TBDT binding sites are generally highly selective for a given ferric-siderophore complex and do not recognize other siderophores (Schalk et al., 2012). Such high selectivity of TBDTs for a given substrate implies that bacteria encode several transporters in their genome: one for

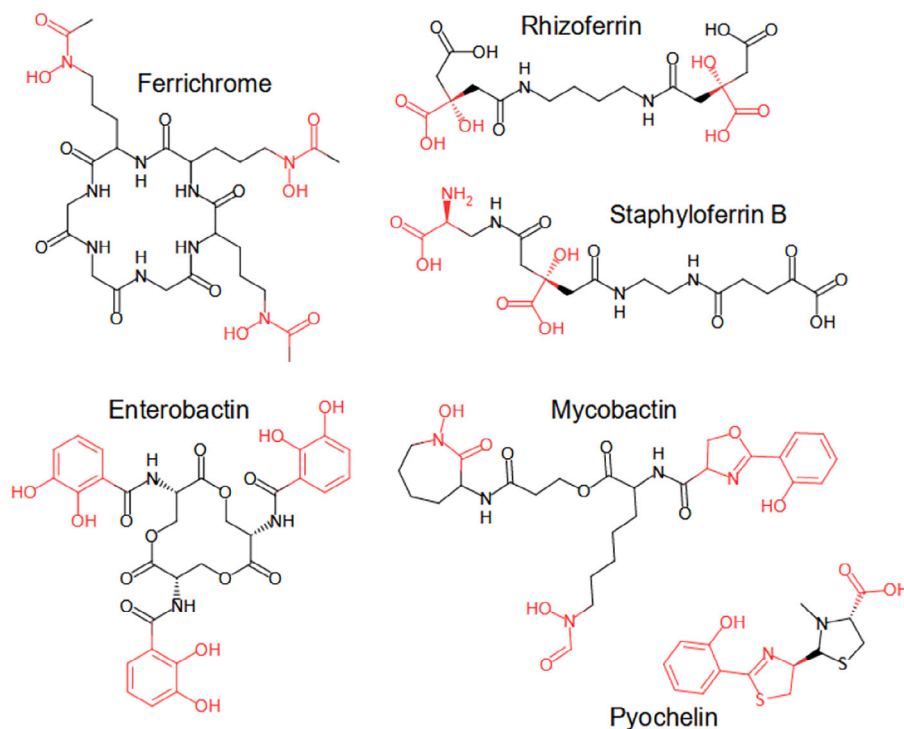


FIGURE 1 Examples of siderophores

each siderophore or structurally similar siderophore used. These transporters also have a region of residues at the N-terminus called the TonB-box, which spans the periplasm and is recognized by the C-terminal domain of the TonB protein (Schalk et al., 2012). Functional and structural data indicate that the interaction of the TonB box with the molecular motor TonB-ExbB-ExbD is necessary to provide the energy for the transport of ferric-siderophore complexes or heme through the TBDTs (Figure 2D) (Braun & Endriss, 2007; Freed et al., 2013; Ogierman & Braun, 2003). Finally, a subfamily of TBDTs has an additional N-terminal domain located in the periplasm called the signaling domain (Figure 2A) (Brillet et al., 2007). This region interacts with ECF (extracytoplasmic factor) anti-sigma factors, located in the inner membrane and coupled to a sigma transcriptional factor that regulates the transcription of the gene encoding the TBDT that interacts with the anti-sigma factor (for a review, see Llamas et al., 2014).

In addition to the acquisition of ferric-siderophores, TBDTs are also involved in other transport, such as the acquisition of cobalamin (vitamin B12) (Pieńko & Trylska, 2020; Rioux & Kadner, 1989), other metals, such as zinc or nickel (Fischer et al., 2016; Lhospice et al., 2017; Schauer et al., 2008), or sugars (Neugebauer et al., 2005), and are entry pathways for certain bacteriophages (Bonhivers et al., 1996; Rabsch et al., 2007) and bacteriocins (Baysse et al., 1999; Behrens et al., 2020; Denayer et al., 2007; Elfarash et al., 2012, 2014).

After passing through the outer membrane, the ferric-siderophore complexes can either deliver iron into the bacterial periplasm or be transported through the inner membrane and deliver the metal into the cytoplasm (Schalk & Guillon, 2013). As will be discussed later, this difference in the mechanism depends on the nature of the siderophore.

TBDTs present in the *P. aeruginosa* PAO1 genome

The genome of *P. aeruginosa* PAO1 encodes 35 TBDTs (Table 1), some of which have only been predicted in silico based on the presence of the N-terminal domain, the plug domain, and the β -barrel structure (Cornelis & Bodilis, 2009).

Most of the TBDTs for which the ligand has been identified are involved in iron homeostasis and there are described in more details in the next sections of these review. TBDTs not involved in iron acquisition include BtuB, predicted to transport cobalamin based on its homology with the *E. coli* BtuB protein (Gudmundsdottir et al., 1989), and OprC, which is induced in the presence of copper and involved in the transport of this metal (Bhamidimarri et al., 2021; Yoneyama & Nakae, 1996). ZnuD, CntO and the proteins encoded by the genes PA1922 and PA2911 are TBDTs involved in the uptake of Zn, and in the case of CntO, it occurs via the metallophore pseudopalmin (Lhospice et al., 2017; Pederick et al., 2015). FpvA,

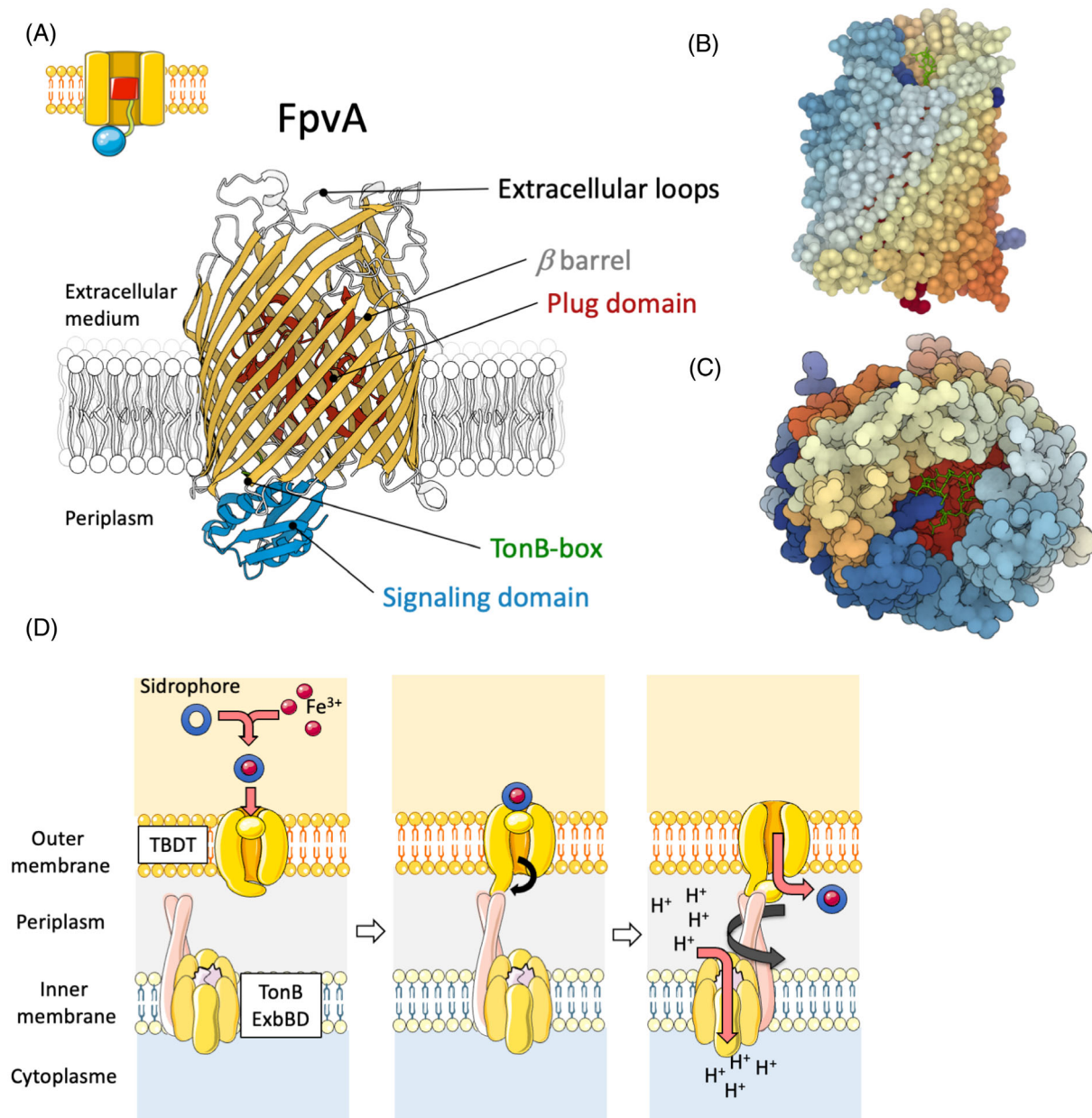


FIGURE 2 (A) General structure of a TBDT: in yellow, the β -barrel with 22 transmembrane β -strands; in red, the plug domain; in green, the TonB-box; and in blue, the signalling domain that allows the interaction with an ECF anti-sigma factor (this domain is absent from TBDTs for which the transcriptional regulation is independent of sigma factors). (B) X-ray structure of FpvA, the ferri-pyoverdine TBDT, view parallel to the membrane (PDB 2O5P) (Brillet et al., 2007). (C) X-ray structure of FpvA, view from the top, with the ferric-pyoverdine in the binding site. (D) Mechanism of TonB-dependent transport. The ferric siderophore interacts with the TBDT binding site. This recognition induces an interaction between the TonB-box of the TBDT and the TonB protein of the inner membrane TonB-ExbB-ExbD molecular motor. The passage of protons through the channel formed by an ExbB pentamer and an ExbD dimer provides energy to TonB, which, in turn, allows energy transfer to the TBDT through the rotation of the TonB proline-rich rigid domain (Celia et al., 2016, 2019, 2020).

FpvB and FptA are the TBDTs of the two siderophores produced by *P. aeruginosa*, pyoverdine and pyochelin. However, these transporters are also the port of entry for bacteriocins, including pyocins S2, S4 (FpvA) and pyocin S5 (FptA) (Baysse et al., 1999; Behrens et al., 2020; Denayer et al., 2007; Elfarash et al., 2012, 2014).

Regulation of TBDT expression

Due to its importance in biological processes and potential toxicity, the intracellular concentration of iron is subject to tight regulation by *P. aeruginosa*, involving precise regulation of the levels of the various proteins involved in heme or iron acquisition. Such regulation

TABLE 1 TBDTs present in the *P. aeruginosa* PAO1 genome

Gene	Name	Ligand	Reference	Status
PA0151	-	-	Cornelis and Bodilis (2009)	Prediction
PA0192	-	-	Cornelis and Bodilis (2009)	Prediction
PA0434	-	-	Cornelis and Bodilis (2009)	Prediction
PA0470	FiuA	Ferrichrome	Hannauer, Barda, et al. (2010a); Llamas et al. (2006)	
PA0781	-	-	Cornelis and Bodilis (2009)	Prediction
PA0931	PirA	Enterobactin, mono catechol	Ghysels et al. (2005); Moynié et al. (2017)	
PA1271	BtuB	Cobalamine	Cornelis and Bodilis (2009)	Prediction
PA1302	HxuA	Haem	Otero-Asman et al. (2019)	
PA1322	PfuA	-	Cornelis and Bodilis (2009)	Prediction
PA1365	-	-	Cornelis and Bodilis (2009)	Prediction
PA1613	-	-	Cornelis and Bodilis (2009)	Prediction
PA1910	FemA	Mycobactins, carboxymycobactins	Llamas et al. (2008)	
PA1922	CirA	Zn	Pederick et al. (2015)	
PA2057	SppR	-	Pletzer et al. (2016)	
PA2070	-	-	Cornelis and Bodilis (2009)	Prediction
PA2089	-	-	Cornelis and Bodilis (2009)	Prediction
PA2289	-	-	Cornelis and Bodilis (2009)	Prediction
PA2335	OptO	-	Cornelis and Bodilis (2009)	Prediction
PA2398	FpvA	Pyoverdine	Poole et al. (1991)	
PA2466	FoxA	Ferrioxamine B, Nocardamine	Llamas et al. (2006); Normant et al. (2020)	
PA2590	-	-	Cornelis and Bodilis (2009)	Prediction
PA2688	PfeA	Enterobactin	Poole et al. (1990)	
PA2911	-	Zn	Pederick et al. (2015)	
PA3268	-	-	Cornelis and Bodilis (2009)	Prediction
PA3408	HasR	HasAp-haem	Smith and Wilks (2015)	
PA3790	OprC	Copper	Bhamidimarri et al. (2021)	
PA3901	FecA	Citrate	Marshall et al. (2009)	
PA4156	FvbA	Vibriobactin	Elias et al. (2011)	
PA4168	FpvB	Pyoverdine	Ghysels et al. (2004)	
PA4221	FptA	Pyochelin	Ankenbauer and Quan (1994)	
PA4514	PiuA	Mono catechol	Moynié et al. (2017)	
PA4675	ChtA	Aerobactin, Schizokinen	Cuiv et al. (2006)	
PA4710	PhuR	Haem	Smith and Wilks (2015)	
PA4837	CntO	Pseudopaline (Zn)	Lhospice et al. (2017)	
PA4897	OptI	-	Cornelis and Bodilis (2009)	Prediction

Note: For the TBDTs with the status of "prediction", no functional data have yet identified the molecules that are transported, and it is only through bioinformatic data that these genes have been identified as encoding TBDTs. TBDTs for which the transcription is regulated by sigma/anti-sigma factors are highlighted in orange, by two component systems in green, by AraC regulators in pink, and by an IclR-type regulator in blue.

mainly involves the transcriptional regulator Fur (Ferric Uptake Regulator), a protein for which homologues can be found in most proteobacteria. In its apo state, Fur exists as stable soluble tetramers in *P. aeruginosa* cells (Pérard et al., 2016). When the concentration of iron increases in the cell, ferrous iron binds to Fur, which induces dissociation of the tetramer and the binding of two Fur dimers to DNA at the Fur-boxes, palindromic sequences of 19 nucleotides (Ochsner & Vasil, 1996; Troxell & Hassan, 2013). This binding prevents the

transcription of the gene located downstream of the Fur-box by blocking the bacterial RNA polymerase (Troxell & Hassan, 2013).

In an iron-deficient environment, the expression of the genes that encode proteins involved in iron acquisition can be activated and different mechanisms and regulators can be involved, depending on the uptake pathway. The regulators consist of ECF anti-sigma/sigma factors, two-component systems, AraC regulators, and an IclR regulator (Figure 3).

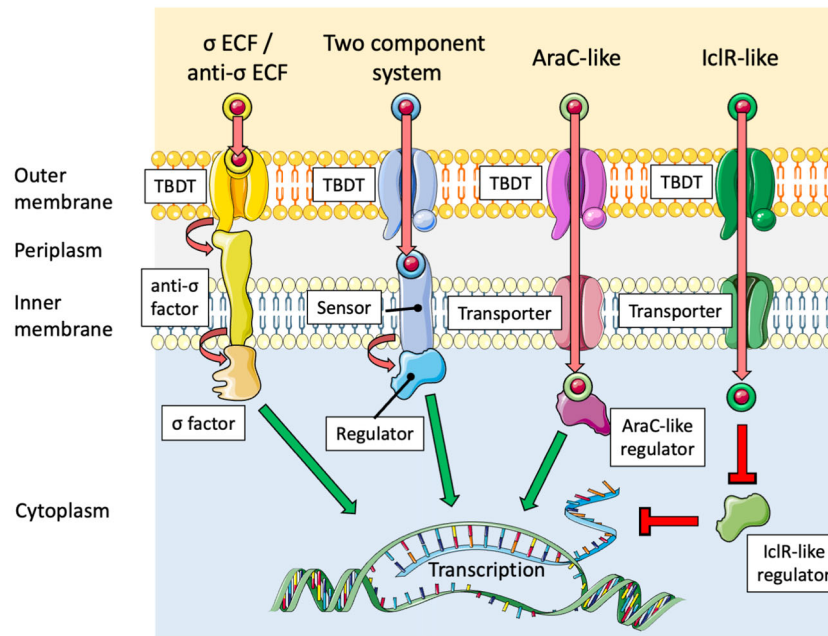


FIGURE 3 The different regulatory systems involved in the regulation of transcription of the genes encoding proteins involved in iron or heme acquisition. For more details, see the text

ECF sigma factors differ from canonical sigma factors in that they are generally associated with an anti-sigma factor, which has the function of sequestering them at the inner membrane, thus allowing the sigma factor ECF to modulate gene transcription according to environmental stimuli (Pinto et al., 2019). In *P. aeruginosa*, eight such regulators are able to induce the transcription of TBDTs involved in iron acquisition and five others are adjacent to TBDTs of unknown function (Llamas et al., 2006, 2008, 2014; Visca et al., 2002). The first step of the molecular mechanism involved in the regulation of gene transcription involving sigma and anti-sigma factors consists of binding of the ferric-siderophore to its specific TBDT on the bacterial surface (Figure 3). This interaction induces a conformation change of the N-terminal signaling domain of the TBDT, allowing an interaction between this domain and the anti-sigma factor and between the TonB box of the TBDT and the TonB protein (Brillet et al., 2007). The interaction between the TBDT and anti-sigma factor allows the dissociation of the sigma factor from the membrane and activation of the transcription of regulated genes (Bishop et al., 2017; Edgar et al., 2014, 2017; Wilson & Lamont, 2000). Using this mechanism, the iron-loaded form of a specific siderophore is detected at the bacterial surface and its presence induces the expression of more of its corresponding TBDT to optimize its uptake.

The two component systems Pfs/PfeR and PirS/PirR are known to be involved in *P. aeruginosa* in the regulation of the transcription of genes encoding TBDTs and other proteins involved in iron acquisition pathways. Pfs/PfeR activates the transcription of *pfeA*

and *pfeE* (encoding a TBDT and an esterase, respectively), both involved in the acquisition of iron by enterobactin, and PirS/PirR activates the transcription of *pirA* (encoding a TBDT), involved in the uptake of iron chelated by mono-catechols (Table 1) (Dean & Poole, 1993b; Gasser et al., 2021; Ghysels et al., 2005; Luscher et al., 2022). Two-component systems are formed by a sensor (Pfs and PirS), located in the inner membrane, and a transcriptional regulator (PfeR or PirR). The sensor has at least one C-terminal histidine kinase, allowing its autophosphorylation and the phosphorylation of the regulator (Hoch, 2000). Once phosphorylated, the regulator activates transcription of the regulated genes. To activate this regulatory system, the ferric-siderophore complex has to enter the bacterial periplasm via its specific TBDT and bind to the TBDT-associated sensor embedded in the inner membrane (Figure 3) (Gasser et al., 2021).

Among the 62 AraC-type regulators predicted to be encoded in the genome of *P. aeruginosa* PAO1, PchR is a cytoplasmic protein that acts as a transcriptional activator of the genes involved in the iron-uptake pathway by the siderophore pyochelin. This regulatory mechanism implies that ferric-pyochelin is transported to the bacterial cytoplasm and interacts with PchR, which then binds to its promoter regions to activate the transcription of genes encoding proteins of the pyochelin-dependent iron-uptake pathway (Figure 3) (Cunrath et al., 2020; Heinrichs & Poole, 1996; Michel et al., 2005, 2007).

The only IclR-type regulator known to be involved in iron homeostasis is FvbR (PA4157). The exact mechanism of such regulation is still uncertain, but it has been

assumed that FvbR acts as a repressor by inhibiting the production of FvbA (the TBĐT of ferric-vibriobactin) and that the presence of vibriobactin bound to FvbR can abolish such repression (Elias et al., 2011).

In these different systems, it is the ferric-siderophore complex that allows the activation of gene transcription by interacting either with the TBĐTs for anti-sigma factors, the sensor in the periplasm in the case of two-component systems, or the cytoplasmic regulators themselves in the case of PchR or FvbR (Figure 3).

IRON ACQUISITION BY ITS OWN SIDEROPHORES, PYOVERDINE AND PYOCHELIN

The pyoverdine-dependent iron-uptake pathway

Pyoverdine is one of the two siderophores produced by *P. aeruginosa*. Indeed, pyoverdines are produced by all fluorescent pseudomonads. They were first called fluorescines (because of their fluorescence) or pseudobactins and were renamed pyoverdine in 1941. Pyoverdines are all composed of a dihydroxyquinoline chromophore linked to an acyl group and a peptide chain of variable sequence and length (Meyer, 2000). The dihydroxyquinoline chromophore preserved among all pyoverdines confers the fluorescent properties and yellow/green coloration in aqueous solution to these molecules. The length and sequence of the peptide moiety varies depending on the pyoverdine (Meyer, 2000). Different isolates of *P. aeruginosa* are able to produce four distinct pyoverdines PVDI, PVDII, PVDIII, and PVDIV, each with a different peptide sequence (Figure 4A), with the PAO1 strain producing type I pyoverdine (PVDI) (Meyer et al., 1997; Ruangviriyachai et al., 2001). The biosynthesis of pyoverdines has been mostly investigated for PVDI produced by *P. aeruginosa* PAO1 (for a review, see Schalk et al., 2020). Pyoverdines are hexadentate ligands with a high affinity for ferric iron (K_a of 10^{32} M^{-1} , $pK_a = 27$ for pyoverdine produced by *P. aeruginosa* PAO1) (Albrecht-Gary et al., 1994). They chelate ferric iron with a stoichiometry of 1 due to the presence of a catecholate and two hydroxamate functions, thus forming an octahedral complex (Albrecht-Gary et al., 1994).

The *P. aeruginosa* PAO1 genome encodes two TBĐTs with high selectivity for ferric-pyoverdine type I (PVDI) uptake, FpvA, and FpvB (Brillet et al., 2007; Ghysels et al., 2004; Poole et al., 1993) (Figure 4B). The dissociation of iron from pyoverdine occurs in the bacterial periplasm, with a mechanism involving the reduction of iron and no chemical modification of the

siderophore and in which the pyoverdine does not enter the cytoplasm at any time (Greenwald et al., 2007; Schalk et al., 2002). After crossing the outer membrane via FpvA or FpvB, the ferric-pyoverdine complex interacts with two periplasmic proteins, FpvC and FpvF, and the ferric ion of the complex is reduced by the inner membrane reductase FpvG (Brillet et al., 2012; Ganne et al., 2017). FpvG is a homologue of FoxB, a reductase involved in iron acquisition by nocardamine (see below). Both FpvG and FoxB belong to a new family of inner-membrane reductases (Josts et al., 2021), and the activity of FpvG is maximal in the presence of the two inner-membrane proteins FpvH and FpvK and the periplasmic protein FpvJ (Ganne et al., 2017). Investigation of the interacting network between these proteins using the bacterial *E. coli* adenylate cyclase two-hybrid system, as well as purification and pulldown experiments, revealed (i) an interaction between the two inner-membrane proteins FpvG and FpvH, (ii) the existence of an FpvJ-FpvC-FpvF periplasmic complex, and (iii) the ability of these five proteins (FpvG, FpvH, FpvJ, FpvC and FpvF) to interact in a membrane complex (Bonneau et al., 2020). These protein interactions probably play a role in the process of iron reduction to obtain the release of iron from the pyoverdine (Bonneau et al., 2020). After iron reduction, the ferrous iron is chelated by the periplasmic protein FpvC, which probably brings the metal to the FpvDE ABC transporter, allowing the passage of ferrous iron into the cytoplasm (Brillet et al., 2012). The structure of FpvC in complex with ferrous iron has been solved and shows an atypical coordination of ferrous iron, with an octahedral geometry involving six histidines, a unique example among iron-binding proteins (Vigouroux et al., 2019). Native mass spectrometry approaches have shown that FpvF can bind apo-pyoverdine and interact with the PvdRT-OpmQ efflux system (Bonneau et al., 2020; Brillet et al., 2012). These results led to the hypothesis that FpvF binds to apo-pyoverdine after iron release and brings it to the efflux pump PvdRT-OpmQ for siderophore recycling into the extracellular medium. Indeed, the deletion of genes encoding PvdRT-OpmQ results in the total inhibition of pyoverdine recycling and its accumulation in the bacterial periplasm (Imperi et al., 2009; Schalk et al., 2002; Yeterian et al., 2010). Newly synthesized pyoverdine is also secreted into the bacterial environment by the PvdRT-OpmQ efflux system (Hannauer, Yeterian, et al., 2010b).

Regulation of the transcription of the various genes encoding the proteins involved in pyoverdine biosynthesis and iron import by pyoverdine involves the anti-sigma factor FpvR associated with the two sigma factors FpvI and PvdS. The binding of ferric-pyoverdine to the FpvA binding site triggers an interaction between the signaling domain of the transporter and the

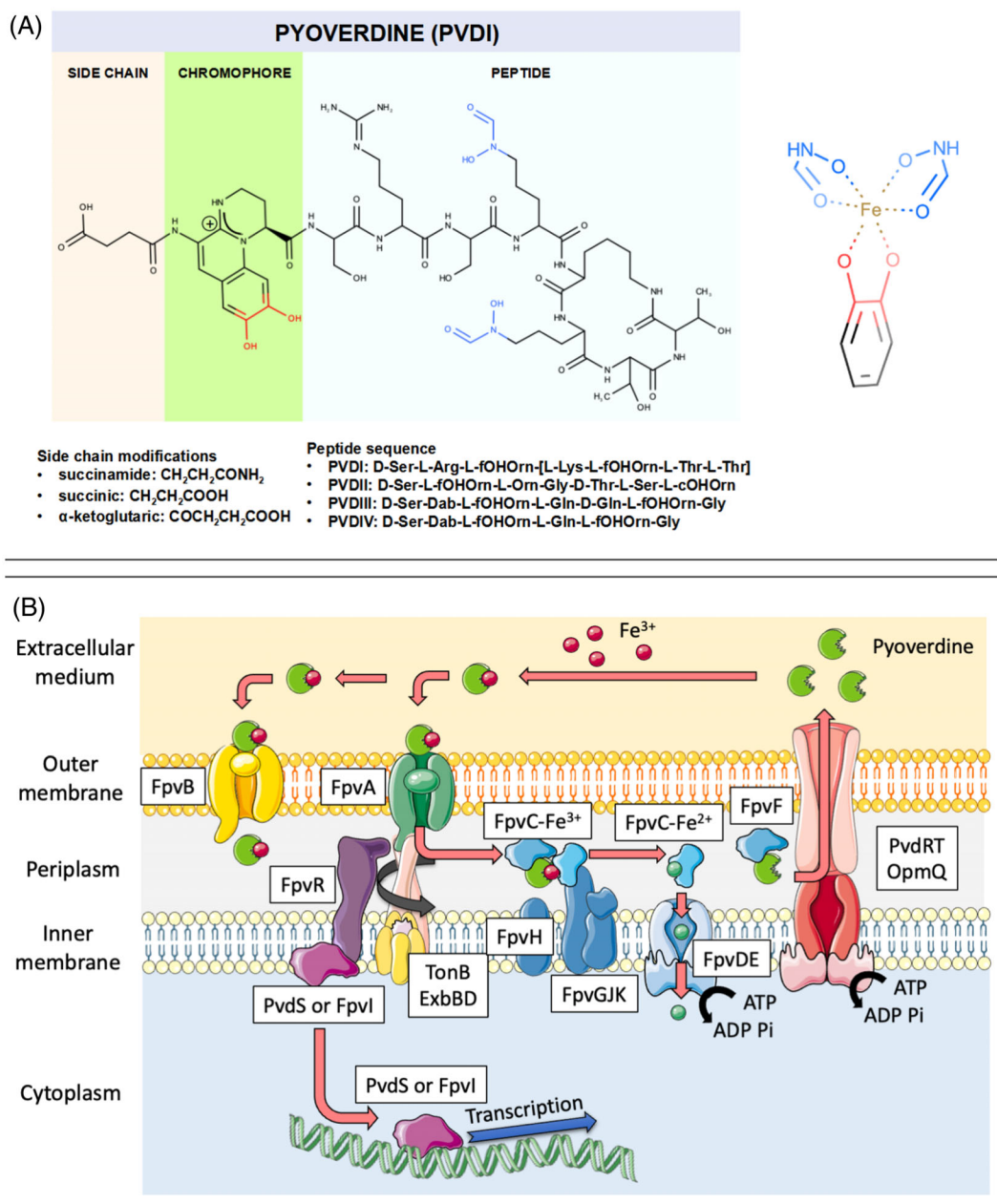


FIGURE 4 (A) Structure of pyoverdines. In blue and red the iron chelating groups. (B) The pyoverdine-dependent iron-uptake pathway. Only the proteins involved in iron uptake by pyoverdine are represented. For the pyoverdine biosynthetic pathway, see the review (Schalk et al., 2020)

periplasmic region of FpvR (Brillet et al., 2007). FpvR then releases the two sigma factors. Fpvl activates the transcription of *fpvA*, and PvdS all the other genes involved in pyoverdine biosynthesis and iron uptake via pyoverdine (Redly & Poole, 2003; Wilson & Lamont, 2000). However, it appeared recently that the regulation of transcription and expression of the genes involved in pyoverdine biosynthesis and uptake is certainly more complex and involves additional regulations with other regulators, including other ECF factors, and LysR regulators (for a review, see Cornelis et al., 2022).

The pyochelin-dependent iron-uptake pathway

Pyochelin (Figure 1), the second siderophore synthesized by *P. aeruginosa*, was first described in 1981 by Cox et al. (1981). It is produced by the condensation of two cysteines and one salicylate. Its biosynthesis has been recently described in detail (Schalk et al., 2020). Pyochelin is not an hexadentate chelator like pyoverdine but a tetradentate ligand that chelates ferric ion through two amines and two alcohols, with a stoichiometry of two pyochelin molecules for one ferric ion

(Brandel et al., 2012; Tseng et al., 2006). This siderophore is often considered to be the “low affinity” siderophore of *P. aeruginosa* (K_a of 10^{28} M^{-2} For iron, $p\text{Fe} = 16.0$) (Brandel et al., 2012) relative to pyoverdine, which has a higher affinity for this metal. Dumas et al., have shown that *P. aeruginosa* produces mainly pyoverdine under severe iron limitation and pyochelin when iron is only moderately limited (Dumas et al., 2013).

FptA is the TBDT that allows the assimilation of ferric-pyochelin in *P. aeruginosa* cells (Ankenbauer & Quan, 1994). The crystallographic structure of FptA in complex with ferric-pyochelin shows that the recognition of the ferric-pyochelin complex by FptA is dependent on the presence of a single pyochelin molecule, providing the tetra-coordination of iron (Cobessi et al., 2004). The remaining bi-dentate coordination can be ensured by another molecule not specifically recognized by FptA, which could be another siderophore, such as cepabactin (Mislin et al., 2006). Recent molecular data based on molecular biology, ^{55}Fe uptake assays, and LC–MS/MS quantification, suggest two different fates for the ferric-pyochelin complex once it is in the bacterial periplasm (Figure 5): the first fraction of the ferric-pyochelin complexes is transported further into the cytoplasm by the inner-membrane permease FptX and the second undergoes dissociation in the bacterial periplasm by an unknown mechanism and the free iron is transported further into the bacterial cytoplasm by the ABC transporter PchHI (Roche

et al., 2022). The reductase and molecular mechanism that allows iron release from pyochelin has not been yet elucidated. The ferric-pyochelin complex transported into the cytoplasm by FptX certainly binds to PchR. Indeed, the transcription of genes encoding proteins involved in iron acquisition by pyochelin in *P. aeruginosa* cells is regulated by PchR, an AraC-type cytoplasmic transcription factor (Cunrath et al., 2020; Michel et al., 2005, 2007). As already mentioned above, binding of the ferric-pyochelin complex to PchR allows an interaction of the regulator with the promoter regions of the pyochelin pathway genes and activates their transcription. Such a scenario involves a small fraction of ferric-pyochelin being transported into the cytoplasm by FptX and interacting with PchR to increase the expression of the proteins involved in iron acquisition by this siderophore, whereas most of the ferric-pyochelin complexes dissociate in the bacterial periplasm to provide iron to the cells.

IRON ACQUISITION BY EXOSIDEROPHORES PRODUCED BY OTHER BACTERIA

The catecholate siderophore-dependent iron-uptake pathways

Enterobactin (Figure 6A) (more rarely called enterochelin) is a tri-catecholate siderophore isolated from *E. coli*,

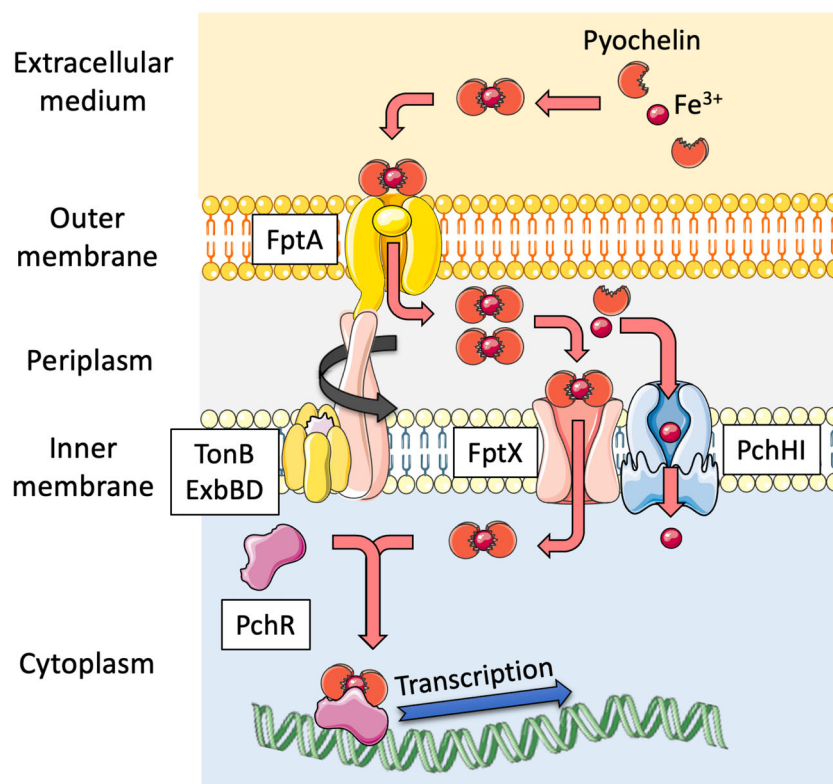


FIGURE 5 The pyochelin-dependent iron-uptake pathway

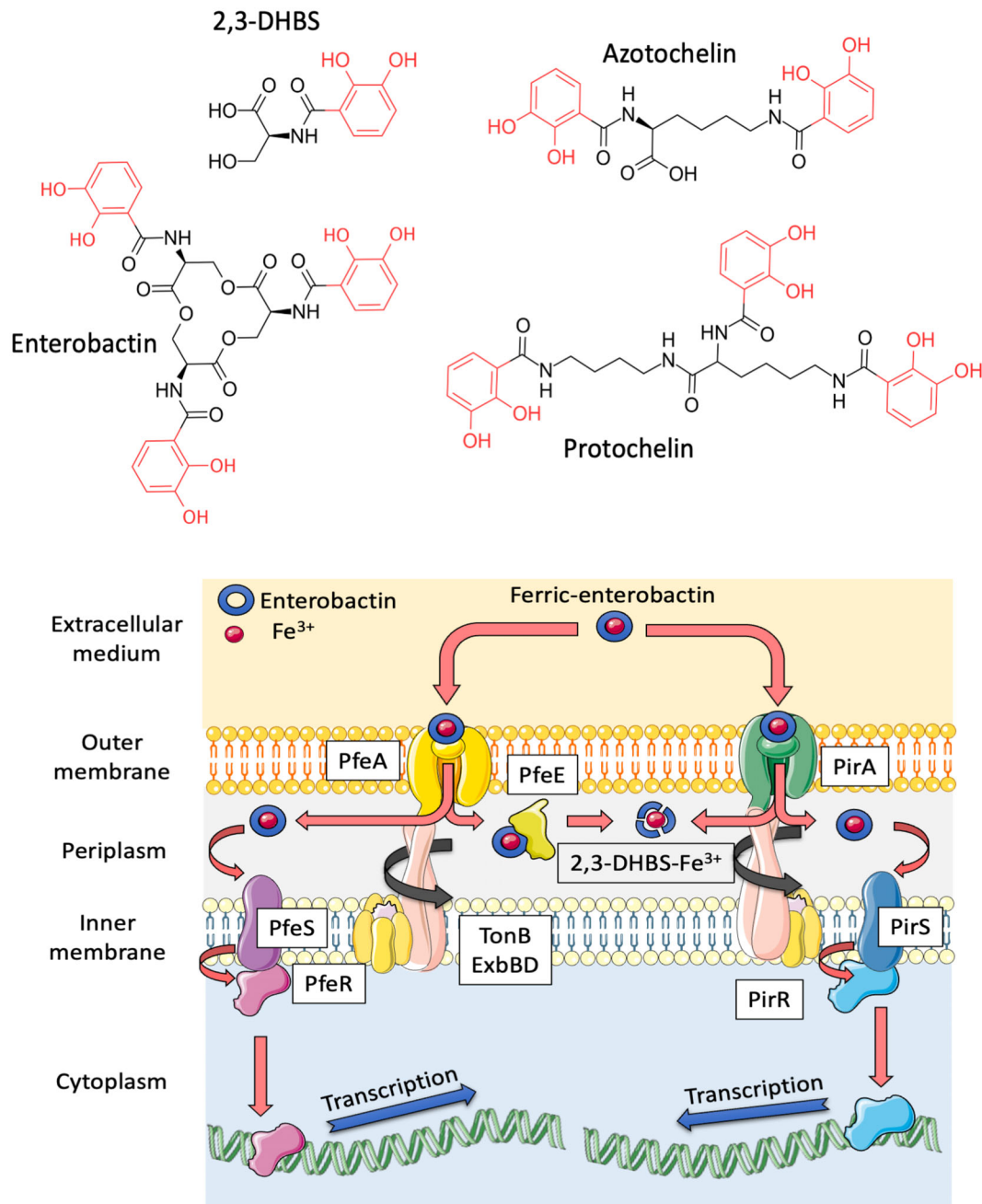


FIGURE 6 (A) Structures of catechol-type siderophores. The catechol functions are shown in red. (B) Enterobactin-dependent iron-uptake pathways

Salmonella typhimurium, and *Klebsiella pneumoniae*. With a pFe(III) of 35.5, enterobactin is currently known as the natural siderophore with the highest affinity for ferric iron (Loomis & Raymond, 1991).

The system of iron acquisition by enterobactin is very well described in *E. coli* (Raymond et al., 2003). In this bacterium, the FepA outer membrane transporter allows uptake of the enterobactin-Fe complex through the external membrane. The complex is then transported through the inner membrane to the cytoplasm via an ABC transporter, FepBCD, and iron dissociation

occurs in the cytoplasm, involving hydrolysis of the siderophore by the esterase Fes and reduction of the metal (Caza et al., 2015). The uptake of iron by enterobactin in *P. aeruginosa* was more recently described and involves a different scenario. Two TBDTs are involved, PfeA and PirA (Dean & Poole, 1993a; Ghysels et al., 2005; Moynié et al., 2019), and iron is then released from enterobactin into the bacterial periplasm by a mechanism involving hydrolysis of the siderophore in three molecules of 2,3-dihydroxybenzoylserine (2,3 DHBS) by the periplasmic esterase PfeE

coupled to an iron-reduction step involving an unidentified reductase (Figure 6B) (Perraud et al., 2018). Indeed, purified PfeE has been shown to hydrolyze ferric-enterobactin into three molecules of 2,3 DHBS when still complexed with ferric iron, and complete dissociation of iron from 2,3 DHBS was only possible in the presence of both PfeE and an iron reducer, such as DTT (Perraud et al., 2018). PfeE plays a key role in iron uptake by enterobactin, as the deletion of *PfeE* renders *P. aeruginosa* unable to access iron via this siderophore (Gasser et al., 2021). The mechanism of iron release from ENT proposed above implies that an inner membrane transporter imports ferrous iron or ferric-(2,-3-DHBS)₃ into the cytoplasm. However, no such candidate has been found near the *pfeA* and *pfeE* genes. *P. aeruginosa* and *E. coli* are both able to access iron from enterobactin but, surprisingly, *P. aeruginosa* hydrolyses the siderophore in the bacterial periplasm to achieve iron release and *E. coli* in the bacterial cytoplasm.

Two other catecholate siderophores, protochelin and azotochelin (Figure 6A), both produced by *Azotobacter vinelandii*, can also be used as exosiderophores by *P. aeruginosa* PAO1 via the enterobactin pathway (Gasser et al., 2016). PfeA structures in complex with the compounds ferric-azotochelin and ferric-protochelin have been recently resolved (Moynié et al., 2019). Protochelin is a hexadentate ligand, similar to enterobactin, whereas azotochelin is a tetradentate ligand, with only two catechol functions. The structure of the ferric-azotochelin-PfeA complex involves ethylene glycol to complete the coordination sphere of ferric-azotochelin and form an octahedral complex, leaving a doubt as to the molecule that would be able to perform this role in a biotic environment (Moynié et al., 2019).

The two genes *pfeS* and *pfeR* are also localized next to the *pfeA* and *pfeE* genes. They encode the two-component system PfeS/PfeR, with PfeS acting as the sensor and PfeR as the transcriptional regulator. *pfeA* and *pfeE* transcription is regulated by the binding of ferric-enterobactin to PfeS after its uptake into the bacterial periplasm (Figure 6B) (Dean & Poole, 1993b). This binding causes the release of the transcriptional regulator PfeR, which activates the transcription of *pfeA* and *pfeE*. *pirA* transcription is not activated by the presence of ferric-enterobactin, probably because this complex is unable to interact with the sensor PirS of the PirS/PirR two-component system, which regulates the transcription of *pirA* (Gasser et al., 2016, 2021).

No genes encoding a reductase, an enzyme able to modify siderophores, or any inner membrane transporters have been found next to *pirA*, *pirR*, and *pirS*, suggesting that the compounds transported by PirA must be mostly ferric iron chelated by mono-catechols, such as iron chelated by plant molecules containing a catechol group (chlorogenic acid, caffeic acid, quercetin, or lutein), or neuromediators, such as L-DOPA,

dopamine, epinephrine, or norepinephrine (see below) (Luscher et al., 2022; Perraud et al., 2022). No hydrolysis of the siderophore is needed for such compounds, as the iron molecule is already chelated by the mono-catechols.

The vibriobactin-dependent iron-uptake pathway

Vibriobactin is a catecholate/hydroxyphenyloxazoline siderophore produced by *Vibrio cholerae* (Figure 7). In addition to three catechol functions, vibriobactin also has two hydroxyphenyloxazoline functions capable of chelating iron. It is likely that several ferric complexes coexist in equilibrium in biotic conditions between this siderophore and ferric iron. Indeed, the structure of ferric vibriobactin highlights a complex in which one of the hydroxyphenyloxazoline functions of the siderophore participates in the chelation of the metal (Li et al., 2012).

P. aeruginosa PAO1 has a TBDT associated with the transport of ferric-vibriobactin, FvbA (Elias et al., 2011), the expression of which is regulated by Fur and the FvbR regulator (PA4157), belonging to the IclR transcriptional regulators. The fate of the ferric-vibriobactin complex after its uptake across the outer membrane is unknown, but the probable cytoplasmic localization of the FvbR regulator tends to suggest that ferric-vibriobactin or one of its metabolites is capable of passing through the cytoplasmic membrane to bind to FvbR. Unlike most siderophore acquisition control systems, FvbR is predicted to be a negative regulator and the presence of vibriobactin prevents it from performing its role, resulting in the transcription of *fvbA* (Elias et al., 2011).

The *fvbA* gene is located directly upstream of a hypothetical cytoplasmic oxidoreductase (PA4155) that could be involved in the dissociation of the ferric-siderophore complex by reducing ferric iron to ferrous iron. The *fehC* gene and the *fehBDG* operon are also found in the vicinity of the outer membrane transporter-encoding gene. These genes were named by analogy with the *E. coli* *feh* genes involved in the acquisition of ferric enterobactin and predicted to encode an ABC transporter (FepBDG), with FepC being the periplasmic binding protein. However, deletion of these genes does not appear to affect ferric vibriobactin transport (Elias et al., 2011).

Mixed alpha-carboxylate/hydroxamate siderophore-dependent iron-uptake pathways

The siderophores aerobactin (synthesized by *E. coli* and a hypervirulent *Klebsiella pneumoniae* strain),

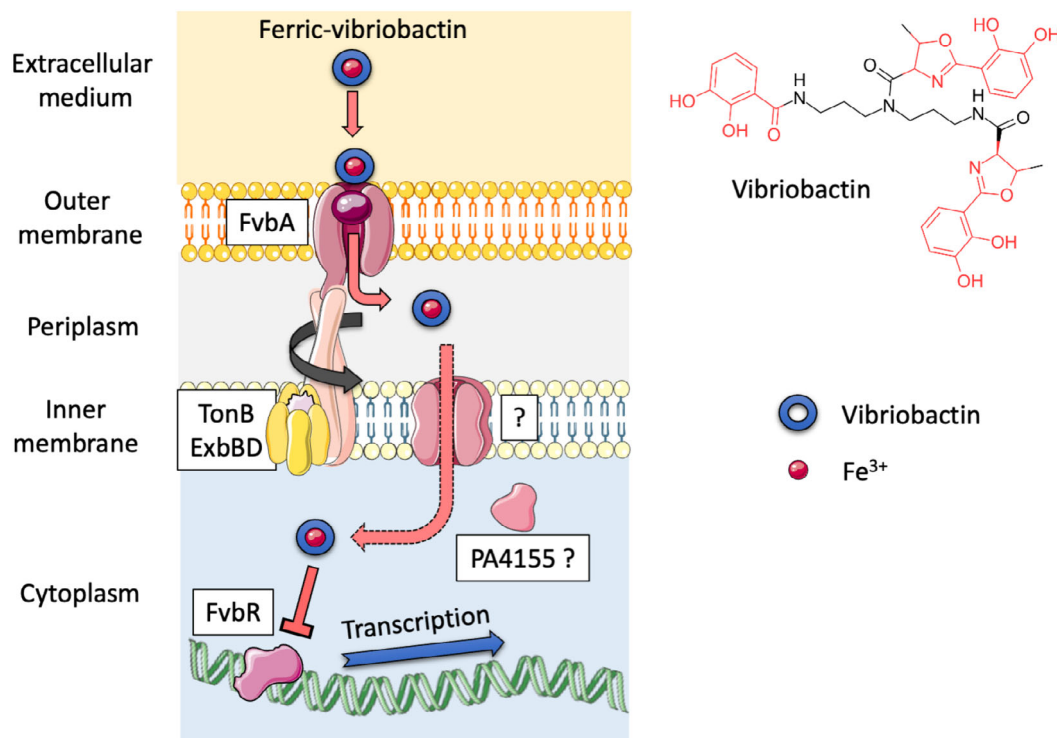


FIGURE 7 The vibriobactin-dependent iron-uptake pathway. Only the proteins for which the biological function has been demonstrated are represented

schizokinen (produced by cyanobacteria such as *Rhizobium* and *Bacillus megaterium*), and rhizobactin (produced, among others, by *Rhizobium meliloti*) are all based on the same molecular frame and chelate iron through two hydroxamate dentate acceptors and an alpha-hydroxycarboxylate group (Akers, 1983; Årstøl & Hohmann-Marriott, 2019; Johnson et al., 1988; Madsen et al., 2015; Storey et al., 2006). They are able to promote the acquisition of iron by *P. aeruginosa* cells and are imported by the TBDT ChtA (Cuiv et al., 2006). Nothing more is known about these iron-uptake pathways concerning the molecular mechanisms involved in the dissociation of iron from these siderophores and whether this step takes place in the cytoplasm or periplasm.

Mycobactin and carboxymycobactin-dependent iron-uptake pathways

Mycobactin and carboxymycobactin siderophores are produced by Mycobacterium and are unusual in that they represent a mixture of molecules substituted by a fatty-acid chain of variable length and position (positions R1 to R4 in Figure 8). Carboxymycobactins are distinguished from mycobactins by the presence of a carboxylic acid (or ester) at the end of the fatty chain (positions R1 to R3 and R5 in Figure 8), making it less aliphatic. Iron-loaded forms of these molecules are

transported across the outer membrane of *P. aeruginosa* cells by the FemA TBDT (Llamas et al., 2008). The *femA* gene is organized into an operon with PA1909, a gene for which the product is predicted to be a homologue of the reductases FpvG and FoxB, involved in the dissociation of iron from pyoverdine and nocardamine, respectively, suggesting a reductive function for the protein encoded by this gene and the dissociation of iron from the siderophore in the bacterial periplasm, as for pyoverdine. There is also an operon predicted to encode an internal membrane MFS transporter directly downstream of the *femA-PA1909* operon that could be involved in the transport of ferrous iron across the inner membrane. The transcription of *femA*, and probably PA1909, is regulated in *P. aeruginosa* by the sigma/anti-sigma system FemI/FemR (Llamas et al., 2008).

IRON ACQUISITION BY EXOSIDEROPHORES PRODUCED BY FUNGI

The hydroxamate siderophore-dependent iron-uptake pathways

P. aeruginosa sometimes shares an ecological niche with fungi, some of which also have iron acquisition systems mediated by siderophores, generally

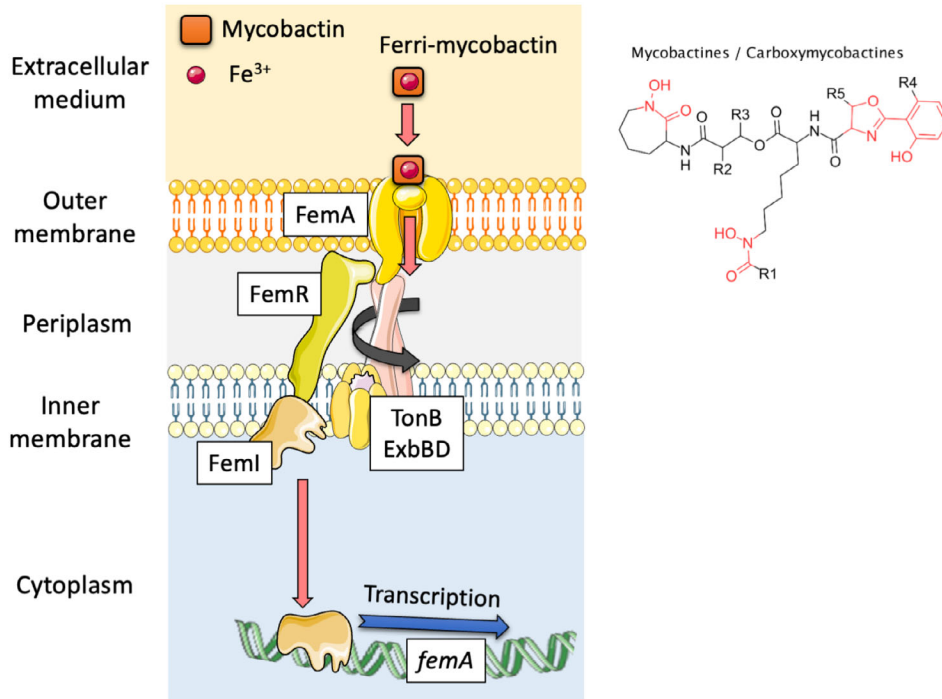


FIGURE 8 Mycobactin-dependent iron uptake. The proteins encoded by PA1909 and the MFS transporter are not represented, as there is no data proving their involvement in iron acquisition by mycobactin or their biological role

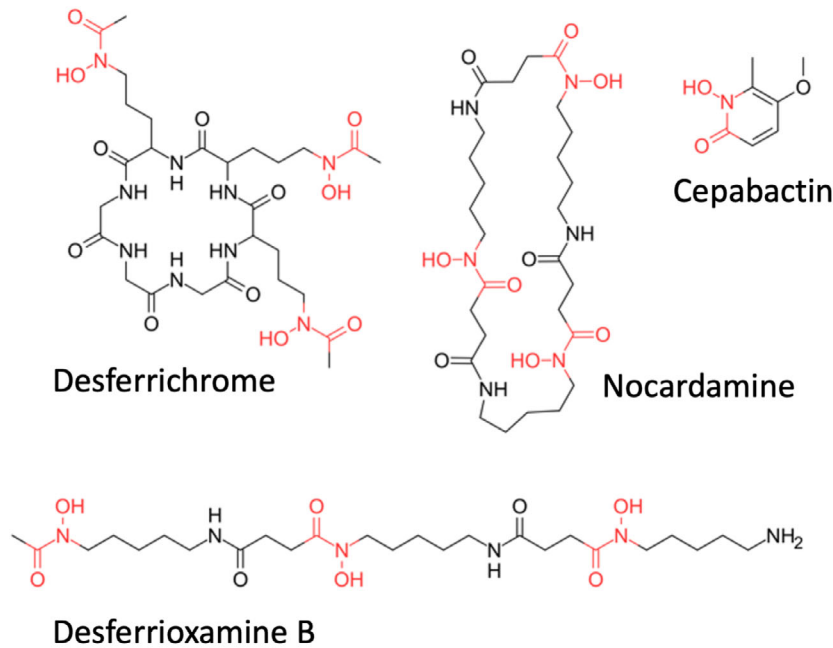


FIGURE 9 Structures of hydroxamate-type siderophores. The hydroxamate functions are shown in red

hydroxamate-type siderophores. Iron acquisition by three different hydroxamate siderophores has been studied in detail in *P. aeruginosa*: one using ferrichrome (pFe = 25.2), one using desferrioxamine B (pFe = 25), and one using nocardamine (also called desferrioxamine E) (Figure 9) (Cuiv et al., 2007; Hannauer,

Barda, et al., 2010a; Llamas et al., 2006; Normant et al., 2020).

Ferrichrome is a siderophore produced by fungi such as *Ustilago*, *Penicillium*, and *Aspergillus*. Its opportunistic use by *P. aeruginosa* is dependent on the TBĐT FiuA and a second non-identified TBĐT

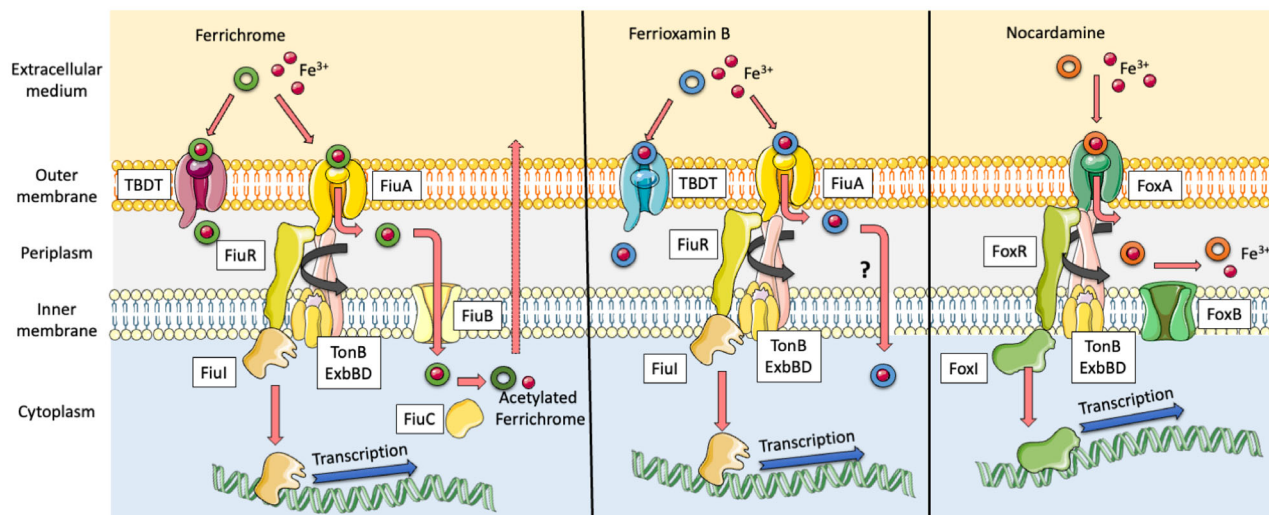


FIGURE 10 Hydroxamate siderophore-dependent iron-uptake pathways

(Figure 10) (Hannauer, Barda, et al., 2010a). After passage across the external membrane, the ferrichrome iron complex diffuses through the cytoplasmic membrane via the permease FiuB (PA0476) (Hannauer, Barda, et al., 2010a). In the cytoplasm, iron is released from ferrichrome by a mechanism involving both reduction by a non-identified reductase and the acetylation of ferrichrome by the cytoplasmic N-acetyltransferase FiuC (PA0478) (Hannauer, Barda, et al., 2010a). This mechanism is very similar to that described previously for *E. coli* (Matzanke et al., 2004) with an acetylation of the desferri-siderophore on the *N*-hydroxyl groups by FiuC. Mass spectrometry analyses have shown a diacetylation of ferrichrome during iron uptake in *P. aeruginosa* (Hannauer, Barda, et al., 2010a) and this modification of the siderophore is probably a mechanism by which cells remove potentially deleterious metal chelators from the cytoplasm. Acetylated ferrichrome can then be excreted into the external environment by an unidentified efflux mechanism. In the extracellular medium, the acetylated desferrichrome reacts with ferric iron and is able to be taken up again by the bacterial cells. Indeed, ferric iron acts as a Lewis acid, hydrolyzing the acetyl group. The transcription of the genes encoding proteins of this pathway is induced by the anti-sigma and sigma factors FiuI and FiuR (Llamas et al., 2006).

Ferrioxamines represent a diverse family of linear siderophores that chelate iron using the hydroxamate function, which can be synthesized by fungi or *Streptomyces*. ^{55}Fe uptake assays using *foxA* mutants showed ferric-nocardamine to be exclusively transported by FoxA, whereas ferric-desferrioxamine B was transported by FoxA and at least one other unidentified TBDT (Normant et al., 2020). The crystal structure of FoxA complexed with ferric-nocardamine or ferric-desferrioxamine B revealed very similar siderophore

binding sites between the two ferric-siderophores (Normant et al., 2020). The mechanism of iron dissociation from ferrioxamines transported by FoxA involves the inner membrane reductase FoxB (Figure 10) (Josts et al., 2021). The X-ray structure of FoxB shows a transmembrane domain harboring two heme groups, certainly involved in electron transfer (Josts et al., 2021). Reduction of iron(III) to iron(II) bound to the siderophore lowers the thermodynamic stability of the complex, which facilitates iron dissociation by proton-mediated exchange or competitive chelation with another iron chelator, such as a periplasmic iron-binding protein. *foxA* and *foxB* transcription is under the regulation of the anti-sigma and sigma factors FiuI and FiuR (Llamas et al., 2006).

Another hydroxamate siderophore that can be used by *P. aeruginosa* is cepabactin, a small bidentate siderophore produced by *Burkholderia cenocepacia* (previously named *Pseudomonas cepacia*). This small siderophore is capable of forming ferric complexes in the presence of pyochelin and is imported into the bacterium by the TBDT FptA in the presence of ferric-pyochelin (Mislin et al., 2006). In this case, the ferric iron is chelated by a molecule of pyochelin, supplying two of the dentate ligand for chelation, and a molecule of cepabactin to complete the hexacoordination of iron (Mislin et al., 2006).

THE CITRATE-DEPENDENT IRON-UPTAKE PATHWAY

P. aeruginosa is able to use citrate as an exosiderophore (Marshall et al., 2009). The ferric-citrate complex is recognized and transported to the periplasm by the TBDT FecA (PA4825) (Figure 11) (Marshall et al., 2009). Ferric-citrate complexes represent a fairly

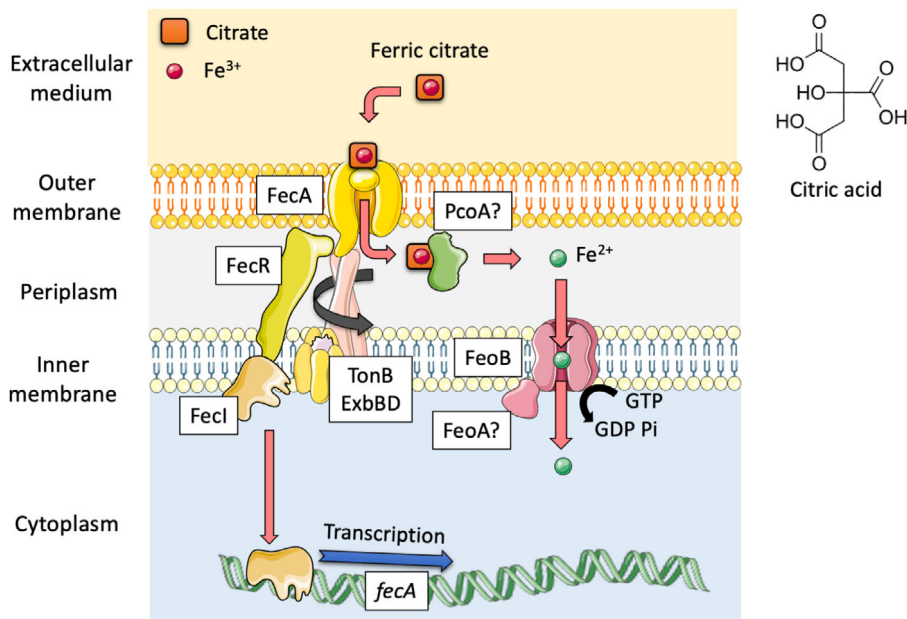


FIGURE 11 The citrate-dependent iron uptake pathway

large category of complexes, with varying stoichiometry, coexisting in the equilibrium state in solution. The complexes sometimes involve water molecules for bonding of the metal and are able to polymerize. It is therefore difficult to determine the stoichiometry of the ferric-citrate complex recognized by the TBDT FecA, although the literature appears to take for granted that the ferric-dicitrate form is recognized. The transcription of *fecA* is regulated by the sigma pair ECF/anti-sigma FecI/FecR (Llamas et al., 2008). Once in the periplasm, it is assumed that the ferric-citrate complex dissociates by a mechanism involving iron reduction by a periplasmic ferredoxin PcoA. Only the ferrous iron migrates through the internal membrane via the inner membrane FeoAB (Marshall et al., 2009).

ACCESS OF IRON VIA MONO CATECHOLS

Recently, it has been shown, that catecholamine neurotransmitters like dopamine, L-DOPA, epinephrine and norepinephrine are able to chelate iron and efficiently transport iron into *P. aeruginosa* cells via TonB-dependent transporters (TBDTs) (Perraud et al., 2022). The TBDTs involved are PiuA and PirA. PiuA exhibits a more pronounced specificity for dopamine uptake than for norepinephrine, epinephrine and L-DOPA, whereas PirA specificity appeared to be higher for L-DOPA and norepinephrine (Perraud et al., 2022). Catecholamines are unlikely to significantly compete for iron with *P. aeruginosa* siderophores pyoverdine and pyochelin. However this does not exclude an important biological role for these molecules under certain conditions,

especially during chronic *P. aeruginosa* infections, for which it is known that spontaneous mutations of the PvdS regulator can cause the absence of pyoverdine secretion without, however, affecting the fitness of the bacteria.

P. aeruginosa is also capable of utilizing plant-derived phenols containing a catechol group (i.e., chlorogenic acid, caffeic acid, quercetin, luteolin) to access iron, and the TBDTs involved are also PiuA or PirA (Luscher et al., 2022). All these mono catechols from mammals or plants induce the transcription and expression of *pirA* when present in the environment of *P. aeruginosa* (Luscher et al., 2022; Perraud et al., 2022). This induction requires the two-component system PirR-PirS.

FERROUS IRON UPTAKE

Unlike iron(III), iron(II) is soluble in aqueous medium, although it spontaneously oxidizes upon contact with oxygen and precipitates in the form of ferric hydroxide. Because of its solubility, iron(II) is believed to diffuse freely via porins across the outer membrane of Gram-negative bacteria. In *Acinetobacter baumannii*, OprG porin has been shown to be involved in the uptake of Fe^{2+} (Catel-Ferreira et al., 2016), and this could also be the case for *P. aeruginosa* (Chevalier et al., 2017). Indeed, the *oprG* gene transcription is induced by high iron levels under anaerobic conditions (Catel-Ferreira et al., 2016). The acquisition of iron(II) probably plays a prominent role during the development of *P. aeruginosa* biofilms, as the base of a biofilm can be

considered to be a microaerobic environment. Phenazine secretion (like pyocyanin) has been highlighted in the literature as a roundabout way of acquiring iron; these molecules are able to reduce ferric iron to ferrous iron (Cornelis & Dingemans, 2013). The uptake of ferrous iron through the cytoplasmic membrane has not been investigated in *P. aeruginosa*. Like many bacteria, *P. aeruginosa* is able to express the conserved FeoABC system, which is assumed to allow the passage of ferrous ions through the inner membrane into the cytoplasm (Lau et al., 2016). Given that the data available for this system in *E. coli*, *S. maltophilia*, *K. pneumoniae*, and many other bacteria are transferable to *P. aeruginosa*, *feoB* could encode an internal membrane porin with a GTPase domain on its cytoplasmic face, *feoA* a small protein for which the role and even localization (internal membrane or cytoplasm) is highly debated, and *feoC* a LysR-type transcriptional regulator capable of binding to iron(II) (Lau et al., 2016).

HEME UPTAKE

Currently, three heme acquisition systems have been described for *P. aeruginosa*: the Phu (*Pseudomonas* heme uptake), Has (heme-acquisition system), and PA1300-1302 systems (Smith & Wilks, 2015) (Figure 12). PA1302 is a gene predicted to encode a TBDT, also known as HxC, and was recently renamed HxA (Otero-Asman et al., 2019), adding to the confusion surrounding this transporter. The Phu and Has systems are very well characterized, whereas the

PA1302 classification within the heme TBDTs is still uncertain. HxA was first identified as a heme TBDT based on its strong homology with the HxC transporter of *Haemophilus influenzae*, which is involved in the import of free heme (Cornelis & Bodilis, 2009). According to recent studies, it appears that HxA is overproduced in the presence of high (20 μ M) concentrations of heme and the transcription of HxA is assumed to be under the control of HxU and HxR, which encode a sigma/anti-sigma factor pair (Otero-Asman et al., 2019). The data concerning the role of HxA in heme homeostasis in *P. aeruginosa* tends, instead, to indicate a signaling role rather than a transport role (Otero-Asman et al., 2019).

The Phu system is composed of the TBDT PhuR, which is able to import free heme into the bacterial periplasm, and proteins encoded by the operon *phuS-TUVW*. *phuR* is overexpressed under conditions of iron deficiency and no specific regulatory system has yet been identified for its transcription. Once heme is imported into the periplasm by PhuR, it binds to the periplasmic binding protein PhuT, which brings it to the inner membrane ABC transporter PhuUV. Once in the cytoplasm, heme binds to the PhuS protein (Smith & Wilks, 2015). HemO interacts with the PhuS-heme complex to degrade exogenous heme to biliverdine IX β and biliverdine IX δ (Barker et al., 2012; Mouriño et al., 2016). The absence of β and δ biliverdins in the cell causes a decrease in the extracellular concentration of the hemophore HasAp of the Has system, suggesting a regulatory role for β and δ biliverdins. The role and localization of the protein expressed by *phuW* is currently unknown, although the gene is predicted to

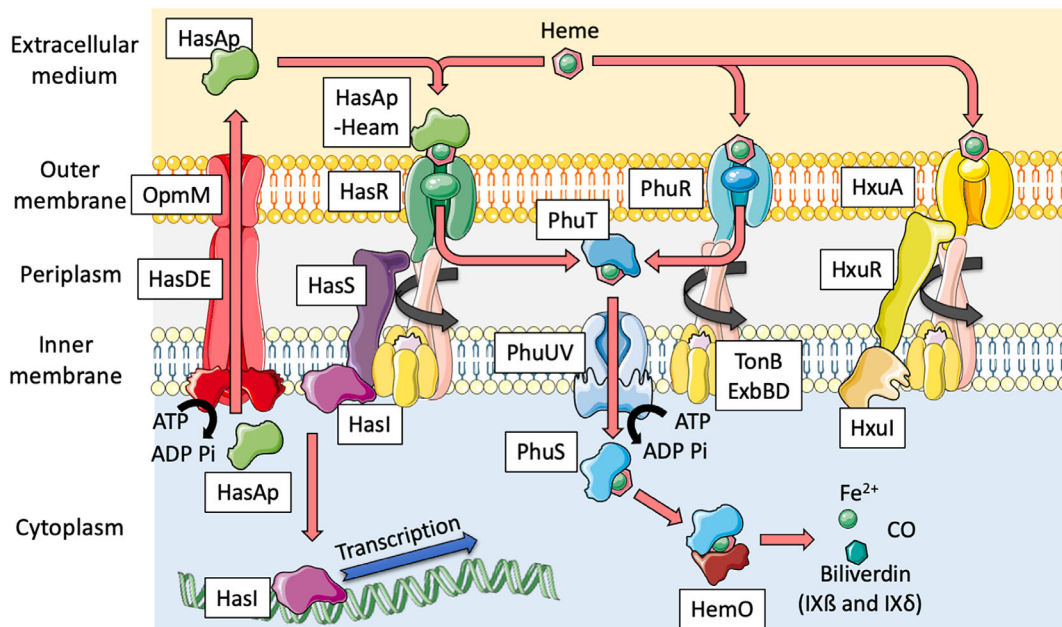


FIGURE 12 Acquisition of heme by *P. aeruginosa*

code for a ChaN-like protein, a hypothetical family of lipoproteins involved in heme acquisition.

The Has system is quite unusual in that it includes the HasAp hemophore, a small secreted protein that scavenges heme from the environment and recognized by the TBDT HasR (Centola et al., 2020; Yukl et al., 2010). This interaction at the level of the outer membrane allows the transfer of heme to the periplasm, where it binds to PhuT and joins the Phu pathway (Dent et al., 2021; Smith et al., 2015; Smith & Wilks, 2015). Unlike the Phu system, the Has system is regulated by the sigma/anti-sigma factors HasI/HasS (Dent et al., 2019). The secretion of HasAp into the extracellular environment is dependent on a HasED-OpmM efflux system (Yukl et al., 2010).

CONCLUSION

The present review highlights the ability of *P. aeruginosa* to use many different siderophores (pyoverdine, pyochelin, and various exosiderophores) to access iron and that this is due to the presence of a plethora of TBDTs in its genome with very different binding specificities. All ferric-siderophore complexes, as well as heme molecules, enter bacteria via these TBDTs. Following entry, the molecular mechanisms that are involved in the release of iron from the siderophores vary widely and are siderophore dependent. In certain cases, this step occurs in the bacterial periplasm and for others, in the cytoplasm, always involving an iron reduction step associated, in some cases, with a chemical modification or hydrolysis of the siderophore.

This ability of *P. aeruginosa* to use multiple strategies and different exosiderophores to access iron, with different associated molecular mechanisms, is certainly an asset for the colonization of complex polymicrobial communities involving a multitude of different species in many different natural and artificial environments. A bacterium able to use the siderophores of a competitor in a strategy of «siderophore piracy» will have a significant advantage in the fight for survival or domination of an ecological niche. Indeed, many studies have shown that iron and siderophores play a significant role in microbiota dynamics (Baishya & Wakeman, 2019; Stacy et al., 2016). A good example of such a complex environment in which several species can coexist is the respiratory system of a patient with cystic fibrosis, where *P. aeruginosa* may come into contact with bacteria such as *Burkholderia cepacia*, *Achromobacter xyloxidans*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Mycobacterium abscessus*, and other atypical mycobacteria, *Haemophilus influenzae*, or pneumococci. In addition to these bacteria, *P. aeruginosa* may also come into contact with fungal pathogens, such as *Aspergillus* sp. or

Scedosporium sp. (Bellis et al., 2019). All these microorganisms are in competition for iron. How the ability of *P. aeruginosa* to use many different exosiderophores to access iron in such a complex environment is still unclear and needs further investigations.

AUTHOR CONTRIBUTIONS

Isabelle Jeanne Schalk: Conceptualization (equal); funding acquisition (lead); project administration (lead); supervision (lead); writing – original draft (equal); writing – review and editing (equal). **Quentin Perraud:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this work.

DATA AVAILABILITY STATEMENT

All data are included in the manuscript.

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