Mini-Review

Siderophore-mediated iron acquisition in *Bacillus anthracis* and related strainsKinya Hotta,¹ Chu-Young Kim,¹ David T. Fox² and Andrew T. Koppisch²

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Recent observations have shed light on some of the endogenous iron-acquisition mechanisms of members of the *Bacillus cereus sensu lato* group. In particular, pathogens in the *B. cereus* group use siderophores with both unique chemical structures and biological roles. This review will focus on recent discoveries in siderophore biosynthesis and biology in this group, which contains numerous human pathogens, most notably the causative agent of anthrax, *Bacillus anthracis*.

Introduction

Iron is a requisite nutrient for the growth and proliferation of bacteria. Unlike higher organisms, which may obtain bioavailable iron from the diet, bacteria must acquire iron from their environment. This presents a challenging problem, as soluble Fe³⁺ in aqueous oxic environments typically exists at attomolar (10^{−18}) concentrations (Harris, 2002). Iron homeostasis in a mammalian host is also tightly regulated; virtually no iron exists in serum that is not bound to haem, iron-storage proteins (ferritin, transferrin, etc.) or as cofactors for various enzymes. Rigorous control of iron availability in mammals is known to serve as a barrier against infection (Andrews *et al.*, 2003; Bullen & Griffiths, 1999). The paucity of iron in serum has driven many pathogenic bacteria to evolve sophisticated means to acquire iron from the host, including the use of high-affinity iron chelates termed siderophores (Boukhalfa & Crumbliss, 2002; Miethke & Marahiel, 2007; Neilands, 1974; Payne & Crosa, 2004; Raymond & Carrano, 1979; Winkelmann, 2002). The efficiency with which this is accomplished by pathogenic bacteria generally parallels their virulence (Byers & Arceneaux, 1998; Griffiths, 1999; Koehler, 2000). As such, the interplay between iron sequestration by the host and acquisition by the pathogen is an important determinant in the establishment of infection. Siderophore biosynthesis by itself is not a *de facto* determinant of virulence but can certainly play a pivotal role in augmenting the pathogenicity of a given microbe (Cendrowski *et al.*, 2004; Dale *et al.*, 2004; De Voss *et al.*, 2000).

Supplementary information, showing the results of BLAST analysis of siderophore uptake and export proteins from *Bacillus thuringiensis*, and a supplementary table, showing predicted genes encoding enzyme homologues for various enzymes involved in siderophore-mediated iron acquisition in *Bacillus* strains, are available with the online version of this paper.

Siderophores of the *Bacillus cereus sensu lato* group

Members of the *B. cereus sensu lato* group are known to produce and utilize two siderophores, bacillibactin (Fig. 1a, boxed structure) and petrobactin (Fig. 2a, boxed structure) (Koppisch *et al.*, 2005; Wilson *et al.*, 2006). Both are considered catechol-based siderophores, in that the metal is bound in the holo-siderophore either partially (petrobactin) or entirely (bacillibactin) by dihydroxybenzoate (DHB) units. Although both molecules are chemically capable of binding and solubilizing iron, genetic, biochemical and virulence studies indicate that they play markedly different biological functions in the bacteria.

Bacillibactin: structure, biochemistry and iron-binding properties

As its name suggests, bacillibactin was identified in culture extracts of *Bacillus subtilis* (May *et al.*, 2001). The siderophore is a cyclic trimer consisting of threonine, glycine and DHB moieties. Like most characterized catecholate siderophores, bacillibactin harbours 2,3-dihydroxylated benzoyl units (2,3-DHB) for efficient chelation of the metal. Typical of most macrolactone siderophores, bacillibactin is biosynthesized via non-ribosomal peptide synthetases (NRPSs), and Marahiel and co-workers have thoroughly characterized the enzymes responsible for its synthesis (Fig. 1a) (May *et al.*, 2001).

The bacillibactin biosynthetic gene cluster is organized in an apparent single operon under Fur regulation (Fig. 1b 1.). The cluster also contains an *mtbH*-like gene whose function remains ambiguous but is frequently associated with NRPS-assisted biosynthesis of aryl-containing natural products (Drake *et al.*, 2007). It also includes an *sfp* gene encoding a 4'-phosphopantetheinyl transferase, essential for proper post-translational phosphopantetheinylation of enzymic domains, a major facilitator superfamily (MFS)-type efflux

Fig. 1. Metabolic route for the biosynthesis of bacillibactin (boxed), and organization of gene clusters involved in the biosynthesis, export and uptake of bacillibactin. (a) The DHB ligand is formed from DHS, which in turn is from the endogenous pentose phosphate pathway (yellow box). (b) (1) Enzymes involved in the assembly of bacillibactin are encoded by the genes of the *entA*–*dhbBCF* cluster. (2) The genes involved in the uptake of bacillibactin originate from the *feuABCD*/*yuiI* gene cluster. (3) Export of bacillibactin is accomplished by an MFS-type transporter encoded by *ymfD*. The genes and gene clusters are annotated with representative ORF numbers, terminator sites (narrow yellow boxes) and the sequences of predicted Fur regulation sites (narrow red boxes). PEP, phosphoenolpyruvate. Abbreviations for enzymic domains (depicted by pentagonal boxes): A, adenylation; C, condensation; ICL, isochorismate lyase; T, thiolation; TE, thioesterase.

transporter and a homologue of *ubiC* which encodes chorismate pyruvate lyase (Siebert *et al.*, 1994), although the role of this gene in bacillibactin biosynthesis remains unclear. Bacillibactin, like related tris-catecholate siderophores, binds iron with an affinity ($K_f = 10^{47.6}$) (Dertz *et al.*, 2006) that is markedly greater than that of the mammalian iron-storage proteins transferrin and ferritin (Dhungana *et al.*, 2004, 2005; Ratledge & Dover, 2000).

Bacillibactin production, uptake and export

Bacillibactin is produced by all examined strains of *Bacillus anthracis* and presumably throughout the *B. cereus sensu lato* group (Koppisch *et al.*, 2008b). While the synthesis of this molecule is widely conserved among *Bacillus* strains, it is not required for the viability of all strains (Cendrowski *et al.*, 2004).

Siderophore uptake in Gram-positive bacteria is facilitated by both membrane-bound substrate-binding proteins and membrane-spanning ABC transporters (Heinrichs *et al.*, 2004). The uptake of bacillibactin in members of the *B. cereus sensu lato* group likely involves FeuA, B and C (Ollinger *et al.*, 2006) as well as the trilactone hydrolase YuiI (BesA) (Miethke *et al.*, 2006, 2008). Genes encoding these four genes in addition to *feuD*, which encodes an ATPase

component of an ABC-type iron-compound transporter, are organized in an operon with a Fur box at its 5' end (Fig. 1b 2.). The FeuABC system has been implicated in the uptake of ferric-bacillibactin in *B. subtilis*, and FeuA has been shown to exhibit nanomolar binding affinity for the ferric-siderophore. YuiI, on the other hand, has been shown to hydrolyse both bacillibactin and ferric-bacillibactin, although it hydrolyses the latter with a 25-fold greater catalytic efficiency. Thus, it has been postulated that YuiI is responsible for liberating iron from its bacillibactin chelate in the cytosol. All of these proteins are conserved in strains within the *B. cereus sensu lato* group (Table 1), and presumably play similar roles in the different strains. Bacillibactin export by these strains likely occurs using the same enzymic machinery as that used by *B. subtilis*, namely the MFS-type transporter YmfD (Fig. 1b 3.) and the transcriptional regulator Mta (Miethke *et al.*, 2008).

Petrobactin: structure, biochemistry and iron-binding properties

Although petrobactin contains catecholate (in addition to an α -hydroxy carboxylate) iron-coordinating moieties, it differs from bacillibactin (as well as all other catechol siderophores) in the hydroxylation pattern of the DHB moieties (Fig. 2a, boxed structure). Petrobactin uses 3,4-

Fig. 2. Metabolic route for the biosynthesis of petrobactin (boxed), and organization of the gene clusters involved in the biosynthesis and uptake of petrobactin. (a) The DHB ligand is formed from DHS, which in turn is from the endogenous pentose phosphate pathway (yellow box). (b) (1) Enzymes involved in the assembly of petrobactin are encoded by the genes from the *asbABCDEF* cluster. (2, 3) Genes involved in the uptake of petrobactin and 3,4-DHB originate from the *fpuA/fhuB* and *fatBCD/fhuC* gene clusters. (c) Genes homologous to iron compound-uptake transporters of unknown substrate specificity.

DHB ligands, whereas the overwhelming majority of catechol siderophores identified to date utilize the 2,3-DHB structural isomer.

Currently, petrobactin and its sulfonated derivatives are the only known siderophores that use this unusual ligand (although free 3,4-DHB is produced by some strains of *B. anthracis* and *Magnetospirillum magneticum*) (Barbeau *et al.*, 2002; Calugay *et al.*, 2006; Gardner *et al.*, 2004; Garner *et al.*, 2004; Hickford *et al.*, 2004; Homann *et al.*, 2009). Petrobactin was initially identified as a siderophore of the petroleum-degrading marine bacterium *Marinobacter hydrocarbonoclasticus* (Barbeau *et al.*, 2002). The first evidence of the production of a 3,4-DHB-containing siderophore by *B. anthracis* was reported by Garner *et al.* (2004), and identification of this molecule as petrobactin was first reported in 2005 (Koppisch *et al.*, 2005). The 3,4-DHB moieties are not only a chemical anomaly in the siderophore world, but have been found to have a significant

biological role in organismal development (discussed below).

Petrobactin is a linear molecule composed of citrate, spermidine and DHB moieties, and its biosynthetic enzymes are distinct from those responsible for cyclic siderophores, and are thus termed NRPS-independent siderophore synthases (NIS synthases). Siderophore production by NIS synthases in other bacteria is relatively widespread. As is the case with petrobactin, NIS synthase-produced siderophores seemingly play a significant role in the virulence of their producers.

Assembly of the three molecular building blocks into petrobactin requires the action of six enzymes, encoded by the *asbABCDEF* gene cluster (Fig. 2a, Table 1) (Cendrowski *et al.*, 2004; Lee *et al.*, 2007; Pfleger *et al.*, 2007). Petrobactin biosynthesis is unusual in that two distinct classes of enzymes, the NRPSs and the NIS synthases, work in concert to provide the final product. Challis and co-

Table 1. Genes found in five representative members of the *B. cereus sensu lato* group that are involved in bacillibactin biosynthesis, uptake and export; petrobactin biosynthesis and export; and uptake of iron compounds of unknown specificityBold type indicates genes upregulated under iron-deficient conditions (Carlson *et al.*, 2009). BB, bacillibactin; PB, petrobactin.

Gene cluster or gene	<i>B. anthracis</i> str. Ames ancestor (AE017334)	<i>B. anthracis</i> str. Sterne (AE017225)	<i>Bacillus</i> <i>thuringiensis</i> str. 97-27 (AE017355)	<i>B. cereus</i> ATCC 14579 (AE016877)	<i>B. cereus</i> ATCC 10987 (AE017194)	Predicted enzyme homologue
Bacillibactin-associated genes						
MFS for BB export	GBAA_1988*	BAS1845–7*	BT9727_1819	BC_1985*	BCE_2066*	YmfD
<i>dhb</i> cluster for BB biosynthesis	GBAA_2368	BAS2204	BT9727_2143	BC_2302	BCE_2398	EntA
	GBAA_2369	BAS2205	BT9727_2144	BC_2303	BCE_2399	DhbC
	GBAA_2370	BAS2206	BT9727_2145	BC_2304	BCE_2400	DhbE
	GBAA_2371	BAS2207	BT9727_2146	BC_2305	BCE_2401	DhbB
	GBAA_2372	BAS2208	BT9727_2147	BC_2306–8*	BCE_2402	DhbF
	GBAA_2373	BAS2209	BT9727_2148	BC_2309	BCE_2403	MbtH
	GBAA_2374	BAS2210	BT9727_2149	BC_2310	BCE_2404	MFS
	GBAA_2375	BAS2211	BT9727_2150	BC_2311	BCE_2405	Sfp
	GBAA_2376	BAS2212	BT9727_2151	BC_2312	BCE_2406	UbiC-like
	GBAA_3863	BAS3579	BT9727_3479	BC_3734	BCE_3767	YuiI
<i>feuABCD/yuiI</i> cluster for BB uptake	GBAA_3864	BAS3580	BT9727_3480	BC_3735	BCE_3768	FeuD
	GBAA_3865	BAS3581	BT9727_3481	BC_3736	BCE_3769	FeuC
	GBAA_3866	BAS3582	BT9727_3482	BC_3737	BCE_3770	FeuB
	GBAA_3867*	BAS3583	BT9727_3483	BC_3738	BCE_3771	FeuA
Petrobactin-associated genes						
<i>asb</i> cluster for PB biosynthesis	GBAA_1981	BAS1838	BT9727_1812	BC_1978	Absent	AsbA
	GBAA_1982	BAS1839	BT9727_1813	BC_1979	Absent	AsbB
	GBAA_1983	BAS1840	BT9727_1814	BC_1980	Absent	AsbC
	GBAA_1984	BAS1841	BT9727_1815	BC_1981	Absent	AsbD
	GBAA_1985	BAS1842	BT9727_1816	BC_1982	Absent	AsbE
	GBAA_1986	BAS1843	BT9727_1817	BC_1983	Absent	AsbF
<i>fpuA/fhuB</i> cluster for PB uptake	GBAA_4766	BAS4424	BT9727_4264	BC_4528	Absent	FpuA
	GBAA_4767	BAS4425	BT9727_4265	BC_4529*	Absent	FhuB-like
<i>fatBCD/fhuC</i> cluster for PB uptake	GBAA_5327	BAS4949	BT9727_4792	BC_5103	BCE_5223	FhuC-like
	GBAA_5328	BAS4951	BT9727_4793	BC_5104	BCE_5224	FatC
	GBAA_5329	BAS4952	BT9727_4794	BC_5105	BCE_5225	FatD
	GBAA_5330	BAS4953	BT9727_4795	BC_5106	BCE_5226	FatB
Unassigned iron compound-transporter genes						
Lone <i>yfiY</i>	GBAA_2255	BAS2099	BT9727_2038	BC_2208	BCE_2283	YfiY (FhuD-like)
<i>feuA/fhuGB</i> cluster	GBAA_3532*	BAS3275	BT9727_3245	BC_3466	BCE_3485	FeuA-like
	GBAA_3533	BAS3276	BT9727_3246	BC_3467	BCE_3486	FhuG-like
	GBAA_3534	BAS3277	BT9727_3247	BC_3468	BCE_3487	FhuB-like
<i>fepC/fhuGD</i> cluster	GBAA_4595	BAS4263	BT9727_4100	BC_4361	BCE_4448	FepC-like
	GBAA_4596	BAS4264	BT9727_4101	BC_4362	BCE_4449	FhuG-like
	GBAA_4597	BAS4265	BT9727_4102	BC_4363	BCE_4450	FhuD-like
Lone <i>fhuD</i>	GBAA_4652	BAS4317	BT9727_4155	BC_4416	Absent	FhuD-like
<i>fepBC/fhuGB</i> cluster	GBAA_5628	BAS5529	BT9727_5060	BC_5380	BCE_5909	FepB-like
	GBAA_5629	BAS5530	BT9727_5061	BC_5381	BCE_5910	FepC-like
	GBAA_5630	BAS5531	BT9727_5062	BC_5382	BCE_5911	FhuG-like
	GBAA_5631*	BAS5532/3*	BT9727_5063	BC_5383	BCE_5912	FhuB-like

*Genes with potentially misassigned GenBank annotations.

workers have provided compelling biochemical evidence for the functions of both of the NIS synthases, AsbA and AsbB (Oves-Costales *et al.*, 2007, 2008). Importantly, they have clearly demonstrated that the type A NIS synthase,

AsbA, specifically catalyses the stereospecific addition of spermidine onto one of the prochiral carboxylates of citrate to yield (3*S*)-*N*⁸-citryl-spermidine (Fig. 2a, 1) (Oves-Costales *et al.*, 2009). The petrobactin biosynthetic pathway

apparently bifurcates at this point by the action of either the NRPS-type protein AsbE, transferring 3,4-DHB from AsbD onto **1** to provide *N*¹-(3,4-dihydroxybenzoyl)-*N*⁸-citryl-spermidine (**2**), or the type C NIS synthase AsbB to afford the bis-amide **3**. Intermediates **2** and **3** converge to **4** by the actions of AsbB and AsbE, respectively, and the biosynthesis of petrobactin is completed by AsbE adding the second molecule of 3,4-DHB to **4**.

Whereas the functions of the AsbABCDE petrobactin biosynthetic enzymes were clearly demonstrated through both *in vivo* and *in vitro* studies, the role of AsbF remained enigmatic. We provided an initial insight into the major metabolic pathway to 3,4-DHB through feeding studies with [U-¹³C]glucose. Based on examination of isotopic enrichment in the catecholate moiety of isolated petrobactin, the early steps of the shikimate pathway were identified as the primary source for 3,4-DHB production (Koppisch *et al.*, 2008a). Shortly thereafter, we and Sherman and co-workers independently characterized AsbF as a dehydroshikimate dehydratase (DHSase) (Fox *et al.*, 2008; Pfleger *et al.*, 2008) for direct conversion of 3-dehydroshikimic acid (DHS) to 3,4-DHB. The action of the NRPS-like enzymes AsbCD is ultimately responsible for the activation of 3,4-DHB for transfer onto the citryl-spermidine framework to complete the assembly into petrobactin.

Although bacillibactin displays a much greater affinity for ferric iron than does petrobactin ($K_f^{\text{bac}} = 10^{47.6}$ vs $K_f^{\text{pet}} = 10^{23}$) (Abergel *et al.*, 2008; Zhang *et al.*, 2009), both have greater affinities towards Fe^{3+} than does transferrin ($K_f = 10^{21}$). Hence, transferrin is the primary source of iron for pathogenic *Bacillus* species during infection. Furthermore, petrobactin removes iron from transferrin at faster rates and with greater efficiency than either enterobactin or aerobactin (Abergel *et al.*, 2008). Undoubtedly, the ability of petrobactin to rapidly extract and solubilize iron from this protein in a microbiologically accessible manner plays a major role in the infectivity of the organism (which can reach a cellular density of 1×10^9 cells ml^{-1} in the mammalian blood stream) (Koehler, 2000). Interestingly, Fe–petrobactin complexes are known to decarboxylate upon prolonged exposure to light (Barbeau *et al.*, 2002), and the photoproduct itself has a greater affinity for Fe^{3+} ($K_f = 10^{24.4}$) than petrobactin itself (Abergel *et al.*, 2008). While this has been postulated to play a role in the life cycle of petrobactin-producing marine bacteria, it presumably does not play an analogous role for the soil-dwelling *Bacillus* strains. Nevertheless, both petrobactin and its photoproduct may be used as xenosiderophores by other members of this species (Abergel *et al.*, 2008).

Petrobactin production and uptake

The production of petrobactin in the *B. cereus sensu lato* group is not exclusive to pathogenic strains (Table 1) (Koppisch *et al.*, 2008b). However, all of the strains that are known to be capable of developing potentially lethal

infections in humans do produce petrobactin as their main siderophore. Thus, while petrobactin production by itself is not a biomarker of virulence in this group, it is a trait that is required for the pathogenesis of this species in mammals (Casadevall, 2006).

Petrobactin uptake is believed to be facilitated by membrane-associated substrate-binding proteins and transporters. Zawadzka and co-workers demonstrated that the substrate-binding proteins FpuA and FatB exhibit a tight binding affinity to both petrobactin (or its photoproduct) and Fe–petrobactin (or its photoproduct), with the latter also exhibiting an ability to bind 3,4-DHB and its bis-liganded ferric iron congener (Zawadzka *et al.*, 2009a). The aforementioned siderophores are also tightly bound by YclQ, a FatB homologue in *B. subtilis*, and orthologues of this protein are found throughout the *B. cereus sensu lato* group (Zawadzka *et al.*, 2009b). Similarly, a survey of the genomes of representative strains from the *B. cereus sensu lato* group reveals the presence of a number of iron-compound transporters whose substrate specificity and function remain unknown (Table 1, Fig. 2c). Thus, it is possible that a number of potential petrobactin-uptake mechanisms exist within any given strain. Despite multiple transporters capable of binding petrobactin, studies by Carlson and co-workers have demonstrated that in *B. anthracis* str. Sterne, FpuA plays a more significant role in petrobactin uptake than FatB in *in vivo* studies (Carlson *et al.*, 2010). Genetic deletion strains of *fpuA* grow more slowly and accumulate petrobactin in the medium to a greater extent than wild-type or *fatB*-deficient strains. Most significantly, spores of the *fpuA*-deficient strain exhibit reduced virulence in a murine model, despite the fact that petrobactin synthesis is not attenuated in this strain. The LD₅₀ of the spores of the *fpuA*-deficient strain is over three orders of magnitude higher than that of wild-type spores, and 10-fold higher than that of a strain deficient in petrobactin production. Some avirulent strains, such as *B. cereus* ATCC 10987 (Table 1), lack petrobactin-synthesis machinery and *fpuA*. Also, an exhaustive comparative analysis of genomes within the *B. cereus sensu lato* group reveals that most non-pathogenic strains lack *fpuA* or *fatB*, with few exceptions (see Supplementary Information). While FpuA seemingly plays a significant role in the virulence of its host strains, the role of FatB is less well understood. However, given that the growth of *fpuA/fatB* deletion strains in iron-deficient media is slowed relative to *fpuA* deletion strains (Carlson *et al.*, 2010), FatB likely plays an auxiliary role in iron acquisition. It is conceivable that the primary role of FatB lies in the transport of 3,4-DHB-liganded iron itself, which is consistent with the observation that the K_d for the substrate $[\text{Fe}^{\text{III}}(3,4\text{-DHB})_2]^{3-}$ is roughly 100-fold lower than that for $[\text{Fe}^{\text{III}}(\text{PB})]^{3-}$ (Zawadzka *et al.*, 2009a). Additionally, unlike the *fat* gene cluster, neither the *asb* nor the *fpu* gene cluster contains a Fur regulatory element, suggesting a closer functional linkage between petrobactin biosynthesis and FpuA-mediated petrobactin uptake (Fig. 2b). Further studies on

these individual enzymes are required before any definitive conclusions can be made. In any case, these studies serve to illustrate the importance of bacterial siderophore uptake in the process of infection and show that the siderophore transport machinery represents another point of intervention for potential therapeutic agents (Ferrerias *et al.*, 2005).

Microbiological roles of bacillibactin and petrobactin

While both petrobactin and bacillibactin solubilize and sequester iron, there is a growing body of evidence which suggests that the two siderophores have distinct biological roles in the bacteria that produce them. The production of petrobactin plays a crucial role in the establishment of *B. anthracis* infection in the mammalian host, while the production of bacillibactin is seemingly far less important in this regard to pathogenic *Bacillus* strains. Genetic deletions of the bacillibactin biosynthetic gene cluster in *B. anthracis* str. Sterne do not affect the growth of the organism in iron-deficient media, nor its observed virulence in a mouse model (Cendrowski *et al.*, 2004). This is partially explained by the observation that the siderophores of *B. anthracis* are produced and accumulate in culture media in a temporally distinct manner (Wilson *et al.*, 2010). Petrobactin accumulates in culture media after 5 h of bacterial outgrowth from spores, which is less than half the time required for bacillibactin accumulation under identical conditions. These studies indicate that petrobactin is used by the bacterium in the early growth stages, such as spore outgrowth, while bacillibactin is not. A petrobactin-deficient mutant strain of *B. anthracis* only grows from spores supplemented with exogenous petrobactin or other iron sources, which is consistent with this hypothesis. Additionally, bacillibactin production by the organism at 37 °C in a CO₂-enriched atmosphere is markedly reduced relative to growth under environmental conditions (Koppisch *et al.*, 2005; Wilson *et al.*, 2010). Wilson and co-workers have reported that growth of vegetative *B. anthracis* str. Sterne at 37 °C without CO₂ supplementation affords a twofold decrease in bacillibactin production relative to cultures grown at 23 °C (10 vs 20 µM, respectively). Bacillibactin is not found to accumulate to a quantifiable amount in cultures amended with 0.4–0.8 % bicarbonate, which is consistent with other CO₂-supplementation analyses (Koppisch *et al.*, 2005). The elevated temperature and carbon dioxide mimic some conditions found in the mammalian circulatory system, and conditions found in the host are known to influence the expression of a number of bacterial virulence traits (Caparon *et al.*, 1992; Koehler *et al.*, 1994; Okada *et al.*, 1993; Shimamura *et al.*, 1985). The suppression of bacillibactin production may reflect an attempt by the organism to conserve metabolic resources from being spent on a molecule that is sequestered by host defences (see below), and thus does not provide a biological advantage under these conditions. Both the temporal production of the two siderophores and the suppression of bacillibactin production by host factors

provide intriguing evidence that the siderophores play discrete biological roles in the life cycle of *B. anthracis*, and presumably other related *Bacillus* strains. While petrobactin is clearly the siderophore that is used by the organism in the outgrowth process and throughout the manifestation of infection, it is postulated that bacillibactin is employed in the later stages of infections when CO₂ concentrations and temperature vary, or in the transition of the vegetative cells back to spores (Wilson *et al.*, 2010).

Siderophores and human health: the role of siderocalin

In general, there is increasing evidence that some siderophores may be considered virulence mediators in the bacterial pathogens that secrete them. Petrobactin synthesis in pathogenic *Bacillus* strains is one of the best illustrations of this concept in microbiology. It has been known for some time that *B. anthracis* strains incapable of producing this siderophore are non-pathogenic, due to the seminal work of Cendrowski *et al.* (2004). While growth of the organism is facilitated by the ability of petrobactin to extract iron from host proteins, growth within the host is further facilitated by the unique ability of the siderophore to evade the endogenous siderophore sequestration protein found in the mammalian immune system, siderocalin (Clifton *et al.*, 2009). Siderocalin has a high affinity for tris-catecholate siderophores, including bacillibactin, but has negligible affinity for petrobactin (Abergel *et al.*, 2006). Further, a subsequent crystal structure of the enzyme bound to bacillibactin suggests that the 3,4-DHB groups of petrobactin may themselves play a role in the occlusion of

Fig. 3. Space-filling model of siderocalin bound to ferric enterobactin based on the X-ray crystallographic structure of the siderocalin–ferric enterobactin complex (protein structure file 3cmp.pdb deposited in Protein Data Bank; <http://www.pdb.org>).

the siderophore from the binding pocket of siderocalin (Fig. 3) (Abergel *et al.*, 2008, 2006). This dichotomy in siderophore binding plays a unique role in the ecology of infection of this organism.

The inability of siderocalin to bind petrobactin allows the siderophore to persist in the bloodstream and to provide a more favourable environment for bacterial growth. Due to its ability to evade siderocalin, petrobactin has been termed a 'stealth' siderophore, and is a critical enabler of virulence in the host. More importantly, the abolition of its production in pathogenic strains has been demonstrated to abolish virulence. To this end, the development of therapeutics that target petrobactin biosynthesis is expected to afford novel means of anti-anthrax treatment and prophylaxis.

Conclusion

Siderophores have long been recognized to be both critically important metabolites and compounds with intriguing metal-binding abilities. In pathogenic bacteria, siderophores themselves are often key virulence mediators. Bacillibactin and petrobactin together provide an interesting example of how iron acquisition not only mediates virulence in a family of pathogens but also influences the development of the organism through its life cycle. With a more thorough understanding of bacterial iron-acquisition strategies, ideally we may garner not only an understanding of the biology of these organisms but also a new insight into how the infections that they cause may be combated.

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