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Identification of a Cluster of Genes that Directs Desferrioxamine Biosynthesis in *Streptomyces coelicolor* M145

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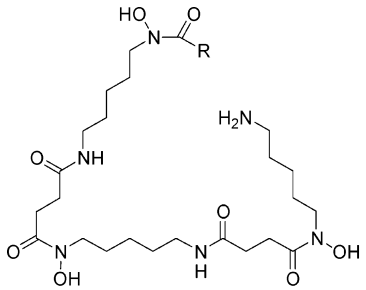
Iron is an essential nutrient for most forms of life, but the availability of ferric iron in aqueous environments such as soil or the intercellular matrix of higher organisms is very low. Consequently, iron acquisition represents a challenging problem for pathogenic and saprophytic microorganisms alike. Many microorganisms tackle this problem by biosynthesizing and excreting high-affinity iron chelators known as siderophores.[1] Once an excreted siderophore has scavenged ferric iron from the environment, the resulting iron−siderophore complex is readsorbed by the cell via a membrane-associated ATP-dependent transport system that often exhibits high substrate selectivity.[2] Several different mechanisms are known for the recovery of ferric iron from the siderophore complex and reduction to ferrous iron for storage and utilization.

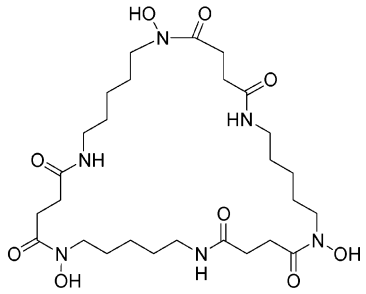
Many siderophores are peptides that are biosynthesized by members of the nonribosomal peptide synthetase (NRPS) multienzyme family, which is also responsible for the biosynthesis of the majority of microbial peptide antibiotics. The enzymology of NRPS-catalyzed siderophore biosynthesis has been intensively studied over the past decade, and the biosynthetic mechanisms for several types of structurally diverse peptide siderophore are now well understood.[3]

On the other hand, several siderophores containing hydroxamic acid-chelating groups are not peptides but consist instead of alternating diamine and dicarboxylic acid building blocks linked by amide bonds. Desferrioxamines, which are secreted by many *Streptomyces* species, are well-known members of this family of nonpeptide hydroxamate siderophores. Among these, desferrioxamine B **1** is of clinical importance because it is marketed as Desferal for the treatment of iron overload in man. The biosynthesis of desferrioxamine B in *Streptomyces pilosus* has been studied by Schupp and co-workers, who showed that the first two steps are decarboxylation of L-lysine to yield cadaverine **4** and subsequent hydroxylation to give *N*-hydroxycadaverine **5** (Scheme 1).[4] How *N*-hydroxycadaverine is incorporated into desferrioxamine, however, has until now been unclear. *Streptomyces coelicolor* A3(2) has been reported to produce a mixture of desferrioxamine G_1 **2** and E **3** under iron-deficient conditions.[5] Here we report the identification of a cluster of genes that directs the production of **2** and **3** in *S. coelicolor*.

Analysis of the recently sequenced *S. coelicolor* genome identified an operon containing four genes (*desA*−*D*) believed to direct desferrioxamine biosynthesis (Figure 1).[6] The *desA* and *desB* genes encode enzymes with similarity to PLP-dependent amino acid decarboxylases and FAD-dependent amine monooxygenses, respectively, and are proposed to catalyze the first two steps of desferrioxamine biosynthesis previously elucidated by Schupp and co-workers.[7] A characteristic inverted repeat sequence for binding of iron(II)-dependent repressor (IdeR or DmdR) proteins is directly upstream of *desA* (Figure 1),[7] consistent with the observation that



1: R = CH_3
2: R = $CH_2CH_2CO_2H$



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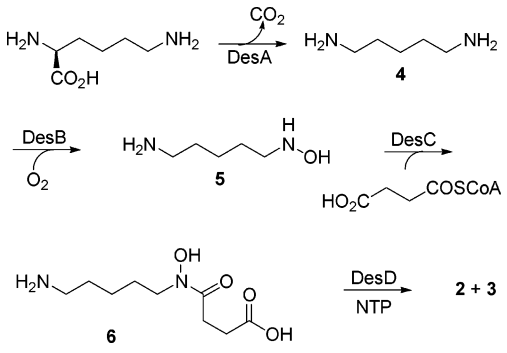
the production of **2** and **3** depends on the concentration of iron in the medium.[5] The protein encoded by *desC* is similar to acyl CoA-dependent acyl transferases. It seems likely that DesC catalyzes the acylation of *N*-hydroxycadaverine **5** to give the hydroxamic acid **6**, which is proposed to undergo nucleotide triphosphate (NTP)-dependent oligomerization to give the trimer **2** and subsequent cyclization to give the macrocycle **3**, both catalyzed by DesD (Scheme 1). In those organisms that also produce **1**, DesC is thought to have relaxed substrate specificity allowing acylation of **5** with acetyl CoA as well as succinyl CoA. It is not easy to rationalize how the production of **1**−**3** could be catalyzed by DesA−D via alternative pathways.

Scheme 1. Proposed Pathway for Desferrioxamine Biosynthesis



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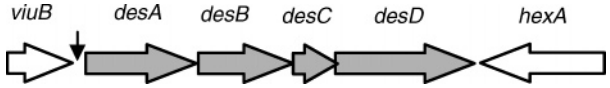

Figure 1. Organization of the *des* operon in *S. coelicolor* M145. The vertical arrow indicates the location of an IdeR binding site.

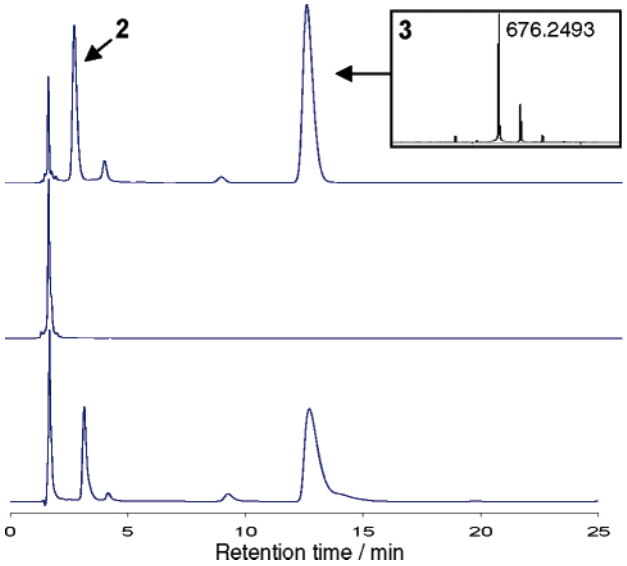

Figure 2. HPLC analysis of desferrioxamine production by *S. coelicolor* M145 (top trace), W2 (middle trace), and W2 + SCC105 (bottom trace), grown under iron-deficient conditions. The inset in the top trace shows the high-resolution mass spectrum of [ferrioxamine E + Na]$^+$ measured for the compound eluting at approximately 13 min from the M145 culture.

To establish whether DesD is required for desferrioxamine biosynthesis, *desD* in *S. coelicolor* M145 was replaced by a cassette containing the apramycin resistance gene using a recently developed PCR-based method,[8] to generate the apramycin-resistant derivative W2. HPLC analysis of the culture supernatant from the M145 strain grown in iron-deficient medium showed that it produces about a 1:2 ratio of **2** and **3,** as well as two other minor desferrioxamines (Figure 2). The major ferrioxamine complex (eluting at about 13 min) was unambiguously confirmed as the ferric complex of **3** (calculated mass = 676.2495) by high-resolution electrospray mass spectrometry (Figure 2 and Supporting Information). Addition of 36 μM iron to the culture medium completely suppressed desferrioxamine production. In contrast, no desferrioxamines could be detected by HPLC analysis of the culture supernatant of the W2 strain grown under iron-deficient conditions (Figure 2). While compound **6** might be expected to accumulate in these cultures neither this nor any alternative intermediates in the pathway could be detected. This result is perhaps not surprising given the known instability of **6** (Castignetti, D. Personal communication). Complementation of the *desD* deletion in W2 with a derivative of the cosmid SCC105,[9] which contains the *des* operon,[6] restored desferrioxamine production under iron-deficient conditions (Figure 2).

These experiments conclusively demonstrate that DesD plays an essential role in desferrioxamine biosynthesis. We propose that DesD catalyzes a similar reaction to that, suggested on the basis of genetic evidence, catalyzed by the homologous enzymes IucA and IucC, which are believed to catalyze condensation of the amino groups of *N*′-hydroxy-*N*′-acyl diamines with the two prochiral carboxyl groups of citric acid in the last two steps of aerobactin biosynthesis.[10] Our results, taken together with the results of Schupp,[4] convincingly establish that the *des* operon directs desferrioxamine biosynthesis in *S. coelicolor*.

Recently, several other gene clusters containing *desD* orthologues have been reported to direct the biosynthesis of structurally diverse siderophores, including alcaligin in *Bordetella pertussis*, rhizobactin 1021 in *Sinorhizobium meliloti*, vibrioferrin in *Vibrio parahaemolyticus*, and structurally uncharacterized siderophores in *Staphylococcus aureus* and *Bacillus anthracis*.[11] Interestingly, many of these bacteria are pathogens, and in several cases the siderophore products of these clusters have been shown to be important for growth under iron-deficient conditions and virulence.

In conclusion, we have identified a desferrioxamine biosynthetic operon in *S. coelicolor* and have demonstrated that *desD* within this operon is essential for desferrioxamine biosynthesis. DesD is a member of a new siderophore synthetase superfamily that shows no sequence similarity to NRPS. Other members of this superfamily are distributed among diverse species of bacteria, including several important pathogens. For many pathogens, iron acquisition via siderophore-mediated uptake pathways is important for virulence. This new superfamily is, therefore, an attractive target for mechanistic investigations, which may facilitate the development of potential new antibacterial agents.

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Supporting Information Available: Procedures for genetic manipulation, and HPLC and mass spectrometric analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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