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Characterization of Siderophore-Mediated Iron Metabolism in *Bordetella pertussis*

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Iron is a fundamental nutritional requirement for virtually all cells, and its assimilation is considered essential for invading pathogenic bacteria to establish infection in the iron-limiting environment of the host (1). Additionally, iron serves as an environmental modulator of the production of certain virulence factors in a number of bacteria. Despite host iron sequestration, mediated primarily by the glycoprotein family of iron-binding transferrins, pathogens multiply successfully in vivo because they express efficient iron scavenging systems in response to decrease iron availability (2). These iron retrieval systems utilize two general strategies: one involving high-affinity iron-chelating soluble siderophores and the other using siderophore-independent cell surface receptor mechanisms allowing iron uptake directly from host sources such as transferrin, lactoferrin, and heme compounds (3).

Bordetella pertussis and *Bordetella bronchiseptica* are Gram-negative bacterial pathogens that cause respiratory diseases in mammals. The native siderophore of both *B. pertussis* and *B. bronchiseptica* is the macrocyclic dihydroxamate alcaligin (Fig. 1) which is expressed in low-iron growth conditions and is under the control of the ferric uptake regulator protein, Fur. The phenotypes of previously isolated *B. bronchiseptica* siderophore deficient mutants suggested that multiple genes were involved in alcaligin biosynthesis; these mutants were adopted as tools to identify the homologous alcaligin

biosynthesis genes in *B. pertussis*. Analysis of one class of mutants led to the identification of the *Bordetella odc* gene which encodes an ornithine decarboxylase catalyzing the conversion of ornithine to putrescine, an essential alcaligin precursor.

Bordetella bronchiseptica transposon insertion mutants BRM1, BRM6, and BRM9 are unable to produce the siderophore. A 4.5 kb *Bam*HI-*Sma*I *Bordetella pertussis* genomic DNA fragment was identified which restored alcaligin production to these siderophore deficient mutants. Genetic complementation analysis using subclones of the 4.5 kb DNA fragment demonstrated that the closely-linked BRM1 and BRM9

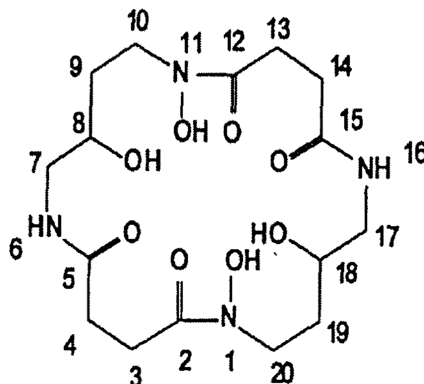

Figure 1. The molecular structure of alcaligin. The dihydroxamate molecule is considered a cyclic dimer of two structural units consisting of 1-amino-4-(*N*-hydroxylamino) -2(*S*)-butanol and succinic acid.

mutations were genetically separable from the BRM6 mutation, and both insertions exerted strong polar effects on expression of the downstream gene defined by the BRM6 mutation, suggesting a polycistronic transcriptional organization of these alcaligin biosynthesis genes.

A putative *Bordetella* promoter was localized to a 0.7 kb *Bam*HI-*Sph*I subregion of the 4.5 kb genomic region. Multiple analyses including nucleotide sequencing, phenotypic complementation of mutants, and protein expression, indicated the presence of three alcaligin system genes: *alcA*, *alcB*, and *alcC*. The deduced proteins AlcA, AlcB, and AlcC share significant primary amino acid sequence similarities with other known microbial siderophore biosynthesis enzymes. Primer extension analysis mapped the transcriptional start site of *alcA* to a promoter region overlapping a proposed Fur repressor binding site (4).

The expression of AlcA, AlcB, and AlcC in *Bordetella* cells was demonstrated by β-galactosidase activity assays and immunoblot analysis using *alc-lacZ* translational fusions; all three *alc-lacZ* fusions exhibited iron-repressible reporter gene expression which was abolished by deletion of the *alcA* promoter/operator region. A *B. pertussis* mutant deleted for the 105 bp *alcA* promoter/operator region was constructed by allelic exchange. Hybridization analysis using gene-specific probes showed that *alc*-specific transcript levels in the mutant were negligible compared with those of the wild-type parent. These results confirmed that *alcA*, *alcB*, and *alcC* are cotranscribed from an iron-regulated control region immediately upstream of *alcA*. Transcript analysis using hybridization probes representing regions downstream of *alcC* demonstrated that *alc* transcription extends approximately 3.6 kb further downstream from the *alcC* coding region. *alcR*, a gene encoding a putative alcaligin gene regulator, is most likely the 3 terminal gene of the *alc* operon and is also transcribed from its own promoter localized immediately upstream of this gene (Fig. 2) (5).

Information based on our experimental data and comparison with other siderophore systems allows the prediction of a hypothetical alcaligin biosynthetic pathway (Fig. 3).

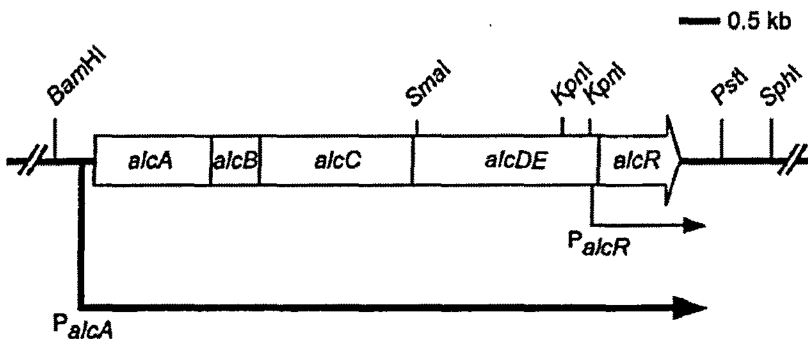

Figure 2. Transcriptional organization of the alcaligin biosynthesis operon. The *alc* operon contains the genes *alcABC*, *alcR*, and *alcDE*. The *alcA* promoter (P_{alcA}) derived transcript is approximately 7.7 kb in size. The gene *alcR* is the last gene transcribed from the *alcA* promoter, and is also transcribed from its own promoter (P_{alcR}).

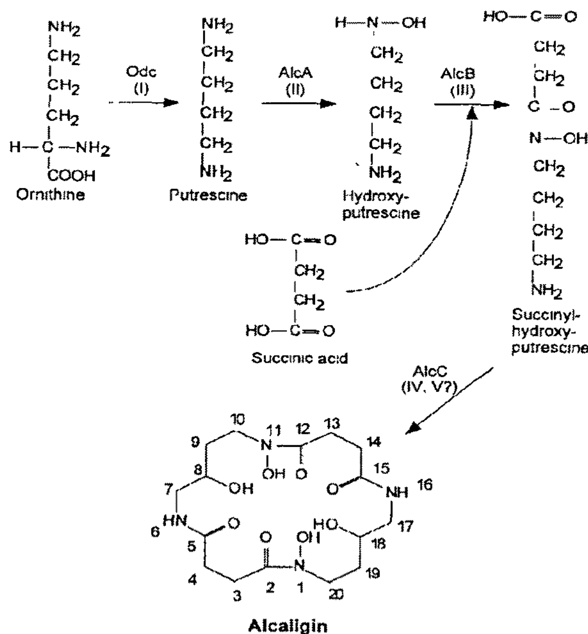

Figure 3. Hypothetical pathway for alcaligin biosynthesis in *Bordetella* spp.

References

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