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Mechanism of Iron Acquisition by the Marine Bacterium *Alteromonas luteoviolacea*

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ABSTRACT

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Marine bacteria exist in an environment that can be extremely iron poor. The levels of iron found in the open ocean are many orders of magnitude lower than that needed to produce normal growth under laboratory culture conditions, yet very little is known about the mechanisms by which marine bacteria may sequester iron. Terrestrial and enteric bacteria have been shown to produce a class of compound called siderophores when grown under conditions of iron limitation. The function of the siderophores is to solubilize and chelate any iron sources, and to make the metal available to the cell.

The marine bacterium *Alteromonas luteoviolacea*, was investigated in an attempt to understand what mechanisms this bacterium may have evolved to adjust to low iron levels in the oceanic environment. Two siderophores, alterobactin A and

B, were isolated from the spent cell culture supernatants of bacteria grown under conditions of iron limitation. The compounds were purified using a combination of solid phase extraction, hydrophobic interaction and gel filtration chromatography. The structures of alterobactins A and B were determined using mass spectral, amino acid and NMR analyses. Alterobactin B is comprised of N^8-(2,3-dihydroxybenzoyl-4,8-diamino-3-hydroxyoctanoyl-seryl-glycyl-aspartyl-(ß-hydroxyaspartyl)-glycyl-(ß-hydroxyaspartate). Alterobactin A is the corresponding depsipeptide formed from the condensation between the serine hydroxyl group and the terminal carboxylate. The lactone ring of alterobactin A is unstable, and undergoes a base catalyzed hydrolysis to form Alterobactin B with a halflife of 11.6 hours at pH 8.0.

Alterobactin A coordinates iron(III) with a stoichiometry of 1:1 and an estimated $ß_{110}$ (the proton independent stability constant) of 10^{48}. Alterobactin B coordinates iron(III) with a 2:1 ligand:iron(III) stoichiometry. The presence of both compounds in the cell culture supernatant is regulated by exogenous iron; the synthesis of both compounds is repressed under high iron conditions. Alterobactin A and B both also function to transport iron into the cells, confirming their function as microbial siderophores.

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