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Involvement of genes of the two-step protein secretion pathway in the transport of the manganese-oxidizing factor across the outer membrane of *Pseudomonas putida* strain GB-I

G.J. Brouwers,* J.P.M. de Vrind, P.L.A.M. Corstjens, and E.W. de Vrind-de Jong

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 R.A. Leiden, The Netherlands

Abstract

Microorganisms can accelerate the rate of Mn^{2+} oxidation by up to five orders of magnitude compared to abiotic Mn^{2+} oxidation. Mn^{2+} oxidation in *Pseudomonas putida* strain GB-1 involves an enzyme incorporated in the outer membrane that oxidizes Mn^{2+} extracellularly. This Mn^{2+}-oxidizing factor has to be synthesized inside the cell and transported across the outer membrane. We used a method known as transposon mutagenesis to generate two mutants that are incapable of Mn^{2+} oxidation because they are unable to transport the Mn^{2+}-oxidizing factor across the outer membrane. However, when cells were lysed, Mn^{2+} oxidation occurred, verifying that transport and not synthesis of the Mn^{2+}-oxidizing factor was affected. Transport of the Mn^{2+}-oxidizing factor was restored when normal sequences obtained from a genomic library of *Pseudomonas putida* strain GB-1 were introduced into the mutant strains. By sequencing the DNA of the disrupted genes of these two mutants it was determined that the affected genes are very similar to the *xpc* gene family of the related species, *Pseudomonas putida* WCS358 and *Pseudomonas aeruginosa*. This gene family is known to be involved in the two-step protein secretion process in Gram-negative bacteria. In one of the two mutants, the disruption occurs in the gene that encodes a subunit of a complex that spans the membrane. In the other mutant the disruption occurs in the *pilD/xcpA* gene, which encodes an enzyme (peptidase) that modifies the subunits that are assembled into this membrane-spanning complex. These results indicate the involvement of a two-step protein secretion pathway in the transport of the Mn^{2+}-oxidizing factor of *Pseudomonas putida* strain GB-1.