Abstract

Investigating new genes required to produce Heat-stable Antifungal Factor (HSAF) in *Lysobacter Enzymogenes*

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Lysobacter enzymogenes strain C3 (LeC3) is a biocontrol agent that produces natural antibiotic compounds to combat microbial pathogens. However, the development of a broad-spectrum antifungal compound like HSAF for agricultural and pharmaceutical applications is hindered by their low yield and complex laboratory synthesis. To increase productivity, it is essential to comprehend how the production of HSAF is regulated in *Lysobacter*. We have previously identified mutants lacking in HSAF production and antifungal activity by transposon mutagenesis. The mechanism behind these mutants' loss of antifungal products is still unknown. The HPLC analysis of metabolite extracts verified that the mutants did not produce HSAF. Antifungal assays confirmed that the mutants did not inhibit *Fusarium graminearum*. The phenol hydroxylase gene (ph), which encodes a protein that is a member of the ferredoxin $NADP^+$ reductase family, is one of the genes that is being studied. VirB10 of the Type IV secretion system family was also studied. We investigated the ph gene's possible physiological role in maintaining redox balance. When exposed to oxidative stress from hydrogen peroxide, methyl viologen, and UV light, the wild-type LeC3 (wtC3) grew faster than the Δph. With the introduction of an antioxidant such as glutathione (GSH), Δph whose metabolism and growth were initially slowed down by oxidative stress, started to have a fast growth. HPLC analysis showed that Δph produced a higher yield of a siderophore called lysochelin. Both wtC3 and Δph development were accelerated by glucose and maltose but slowed by glycerol. TEM and SEM analysis showed pili were barely present in the VirB10 mutant, while wtC3 had a significant number of pili. Our findings suggest that the ph gene may influence redox processes necessary for the cyclization of polycyclic rings of HSAF by participating in electron transfer via reduced NADPH. VirB10 likely participates in constructing the pili that create surface contacts for extracellular signals, to which the *Lysobacter* cells would respond by producing HSAF. We are currently using tools in biochemistry, genetics, and chemical biology to obtain more evidence for the function of these new genes in HSAF production.