

Abstract

ROLE OF *WAAL* AND *UMUDC* IN *ERWINIA AMYLOVORA* EA1189 IN OXIDATIVE STRESS AND ULTRAVIOLET RADIATION SURVIVAL

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Bacteria are exposed to many stresses throughout their life cycle, including ultraviolet radiation (UV) radiation and oxidative stress. Oxidative stress and ultraviolet radiation were focused on specifically because *Erwinia amylovora* has been observed to induce an oxidative burst in host plants, and UV radiation stress was tested because few studies to date have explored the role of UV sensitivity and virulence. A forward genetics approach was used to identify *E. amylovora* Ea1189 gene mutations that resulted in an increased sensitivity to hydrogen peroxide. Of the mutants identified, further study focused on one mutant with a defective *waaL* gene, which is responsible for attaching O-antigen to the lipopolysaccharide (LPS) layer. Other studies have shown that deficiencies in the LPS layer can lead to different phenotypes including decreased virulence, decreased motility, and increased sensitivity to antibiotics. Prior to the work discussed here, a relationship between a truncated LPS layer and increased sensitivity to hydrogen peroxide had not been discovered. Complementation of the *waaL* gene on the plasmid pMCB3 restored the mutant to near WT levels in hydrogen peroxide sensitivity as well as the other phenotypes mentioned. A reverse genetics approach was used to study the response of *E. amylovora* Ea1189 to UV radiation. When compared to other Gram-negative bacteria, *E. amylovora* had a higher survival and mutability rate. Survival was reduced in an *umuDC* knockout strain, whose gene product is responsible for mutagenic DNA repair. Mutability was greatly reduced in the *umuDC* knockout strain, but both phenotypes were restored when complemented with plasmids pJJK25 and pJJK27 which carry the *umuDC* homolog *ruLAB*, and carry *umuDC* respectively.