

**ATPase activity of RecD is essential for growth of the Antarctic
Pseudomonas syringae Lz4W at low temperature**

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Abstract

RecD is essential for growth at low temperature in the Antarctic psychrotrophic bacterium *Pseudomonas syringae* Lz4W. To examine the essential nature of its activity, we analyzed the wild-type and mutant RecD proteins with substitutions of important residues in each of the seven conserved helicase motifs. The wild type RecD displayed DNA dependent ATPase and helicase activity *in vitro*, with the ability to unwind short DNA duplexes containing only 5'-overhang or forked ends. Five of the mutant proteins, K229Q (in motif I), D323N and E324Q (in motif II), Q354E (in motif III), and R660A (in motif VI) completely lost both ATPase and helicase activities. Three other mutants, T259A in motif Ia, R419A in motif IV, and E633Q in motif V exhibited different degrees of reduction in the ATPase, but had no helicase activity. While all RecD proteins had DNA binding activity, the mutants of motif IV and V displayed reduced binding and the motif II mutant showed higher binding to ssDNA. Significantly, only RecD variants having *in vitro* ATPase activity could complement the cold sensitive growth of the *recD*- inactivated strain of *P. syringae* at 4°C. These results suggest that the requirement of RecD at lower temperature lies in its ATP-hydrolyzing activity.