Effects of the L54Q Substitution on the RecA Protein of *Herbaspirillum seropedicae*

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RecA is a multifunctional protein that plays a central role in DNA repair in bacteria. In the presence of single-stranded DNA and ATP, RecA forms a filament which induces LexA autocleavage, triggering the SOS response. In addition, ATP hydrolysis enables the RecA filament to perform DNA strand exchange and consequently homologous recombination. The aim of this work was to determine the effect of the L54Q mutation in the MAW domain of the *H. seropedicae* RecA protein (HsRecA) on its activities. The *recA* gene of *H. seropedicae* bearing the mutation was cloned into the expression vector pET28a and the recombinant protein was over-expressed and purified. The mutant protein was capable of binding ATP, but the ATPase and DNA strand exchange activities were lost. To determine the effects of this substitution on the cell survival, the expression of either HsRecA L54Q or HsRecA proteins was induced in *E. coli* XL1Blue (*recA1*) by IPTG from a *taq* promoter and the cells were challenged with methyl methanesulphonate (MMS), a DNA methylating agent that induces SOS response. Both HsRecA and HsRecA L54Q expressed from the *taq* promoter complemented partially the *E. coli* XL1Blue *recA*$^-$ phenotype indicating that the mutant RecA protein retains its capacity to trigger the SOS response *in vivo*. Full complementation was attained only when HsRecA was expressed from its own promoter probably due to induction of appropriate levels of HsRecA expression. Together, the results suggest that the capacity of inducing the SOS response is not dependent on the ATPase activity of the RecA protein in *H. seropedicae*.

Palavras Chaves: DNA repair, *Herbaspirillum seropedicae*, Homologous recombination, RecA protein
Supported by: CNPq/MCT