TÍTULO: ROLE OF THE ClpP PEPTIDASE IN *Enterococcus faecalis* STRESS RESPONSES

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Resumo:

The Gram positive pathogen *Enterococcus faecalis* ranks among the leading agents that cause severe nosocomial infections. Its capacity to survive under adverse conditions includes prolonged starvation, exposure to detergents and commonly used antimicrobial agents. To survive environmental stresses, cells synthesize special proteins, including chaperones and proteases that prevent accumulation of abnormal proteins. The ClpP peptidase is a serine protease that normally degrades small peptides. However, in association with a partner Clp ATPase, ClpP forms a functional complex that specifically targets damaged and misfolded proteins. Therefore, ClpP performs important housekeeping functions and prevents the accumulation of altered proteins that might be toxic to the bacteria under stress conditions. In this study, we evaluated the biological significance of ClpP in *E. faecalis*. A markerless system was used to inactivate the *clpP* gene in the laboratory strain OG1RF. The ability of the Δ*clpP* mutant to grow under different stress conditions, to form biofilms on microtiter plates, and to kill *Galleria mellonella* was evaluated. While OG1RF was able to grow at 46°C and 50°C, the Δ*clpP* mutant grew at 46°C but not at 50°C. No differences between mutant and wild type strains were observed when strains were grown in media containing 5% NaCl, 0.02% SDS, 1.5% H_2O_2, 10% NaOCl, or under acidic (pH 5) or alkaline conditions (pH 9). Unexpectedly, the Δ*clpP* mutant grew significantly better in the presence of sub-inhibitory concentrations of vancomycin, or in two different cephalosporins (cefotaxime and cefuroxime). Compared to the parent strain, Δ*clpP* showed increased capacity to form biofilms when grown in BHI supplemented with glucose. The Δ*clpP* strain was able to kill *G. mellonella* as efficiently as the parent OG1RF strain, suggesting that ClpP may not be required for the virulence of *E. faecalis*. In conclusion, our findings revealed that, with the exception of thermotolerance, ClpP does not appear to have an essential role in the stress responses of *E. faecalis*. Work is underway to identify alternative pathways that compensate for the loss of *clpP* in *E. faecalis*.

Palavras-chave: stress response, mutants, clpP peptidase, biofilm, *Enterococcus faecalis.*

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