

Molecular study of genes involved in virulence regulatory pathways in *Bacillus anthracis* vaccine strain "Carbosap"

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SUMMARY

This study investigated the genetic bases of attenuation in the *Bacillus anthracis* vaccine strain "Carbosap" used in Italy against anthrax in cattle and sheep. Twelve genes involved in virulence regulatory pathways underwent sequence analysis in comparison with a *B. anthracis* virulent strain.

KEY WORDS: *B. anthracis*, Carbosap, Virulence, pXO1, pXO2, PCR, Sequencing

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INTRODUCTION

Bacillus anthracis, the etiological agent of anthrax, is a Gram-positive, spore-forming, rod-shaped bacterium. The major virulence factors of *B. anthracis* are encoded on two virulence plasmids: pXO1, 182 kb in size (Koehler, 2002), and pXO2, 96 kb in size (Mock & Fouet, 2001). Plasmid pXO1 codes for a tripartite toxin consisting of three proteins, called protective antigen (PA), edema factor (EF) and lethal factor (LF). Plasmid pXO2 encodes for the *cap* operon involved in the synthesis and depolymerization of the capsule. In addition, both plasmids carry regulatory genes that control expression of the toxin and capsule genes: *atxA* and *pagR* on pXO1 and *acpA* and *acpB* on pXO2 (Bourgogne, Drysdale *et al.*, 2003). For anthrax virulence to

be fully manifested, both plasmids must be present; strains lacking either of the plasmids are avirulent or significantly attenuated in most animal hosts. Traditional vaccine strains used against animal anthrax are non-toxinogenic and encapsulated (pXO1/pXO2+), or toxinogenic and non-encapsulated (pXO1+/pXO2-). However, atypical *B. anthracis* strains have also been identified that contain both plasmids but are markedly less pathogenic than the fully virulent strains (Uchida, Sekizaki *et al.*, 1985); (Makino, Cheun *et al.*, 2001). The cause of the lack of virulence of these strains is still unknown.

In Italy, an atypical *B. anthracis* vaccine strain named "Carbosap" is used for anthrax prophylaxis in cattle and sheep. Although it carries both plasmids it shows a much lower virulence in animals when compared with *B. anthracis* virulent strains (Adone, Pasquali *et al.*, 2002).

To evaluate whether attenuation of the *B. anthracis* vaccine strain "Carbosap" was due to lack of virulence genes or genetic differences in their sequences, a previous study analyzed the structural genes encoding for the major virulence factors EF, LF, and PA, in comparison with a *B.*

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