Título: ***BACILLUS SUBTILIS*** **SPORES AS CARRIERS FOR BIOBALLISTICS**

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Resumo:

Bacillus subtilis spores can be used as vaccine vehicles capable of displaying heterologous proteins on its surface. Recent studies have found that the spore surface has hydrophobic properties and is negatively charged, providing a suitable platform for adsorption of proteins, enzymes and viral particles. Nevertheless, no study was performed with coating spores with plasmids or assessing their potential as microparticulated delivery of DNA vaccines. In this work, we demonstrated for the first time that *B. subtilis* spores can adsorb plasmids under specific conditions and be used as vaccine carriers for bioballistic (*gene gun*) delivery. Different cationic reagents were tested and an optimized protocol was determined after one-step treatment with vesicular fragments of the cationic lipid DODAB. In addition, we demonstrated that spores can be loaded into biolistic cartridges for *gene gun* administration and showed efficient results for *in vitro* and *in vivo* transfections. Using a DNA vaccine (pgDE7h) encoding the E7 protein of the human papillomavirus type 16 (HPV-16) and the glycoprotein D (gD) of the herpes simplex virus type 1 (HSV-1) as a model, mice immunized with two doses of spores-coated cartridges elicited E7-specific cellular responses and increased specific-IgG titers compared to mice immunized with gold microparticles commonly used for this method. Notably, *B. subtilis* spores are considered safe and much less expensive in regard to gold. Indeed, purified *B. subtilis* spores can be obtained through very simple and fast techniques using relatively inexpensive medium and exhibiting great relevance in future laboratorial and industrial applications. Together, these data indicate that *B. subtilis* spores represent a simple and effective alternative for gene delivery, particularly for DNA vaccines.

Palavras-chave: *Bacillus subtilis,* spores, adsorption, carriers, *gene gun*.

Agências de fomento: CAPES e FAPESP