TITLE: *Bacillus* endospores strains as potential probiotics against heat-labile toxin (LT) from Enterotoxigenic *Escherichia coli* (ETEC)

AUTHORS: NASCIMENTO, R. S.; SANTOS, A.; CAMPOS, L. C. G.; RODRIGUES, J. F.; FERREIRA, L. C. S.; LUIZ, W. B.

INSTITUTION: UNIVERSIDADE ESTADUAL DE SANTA CRUZ, ILHÉUS, BA (CAMPUS SOANE NAZARÉ DE ANDRADE, RODOVIA JORGE AMADO, KM 16, BAIRRO SALOBRINHO CEP 45662-900, ILHÉUS-BAHIA)

ABSTRACT:

Bacterial spores have been proposed as vehicles to display heterologous proteins for the development of mucosal vaccines, biocatalysts, bioremediation, diagnostic tools, and biological control. Two approaches were described to display proteins on the spore surface: (1) a recombinant approach, based on the gene fusions construction between DNA molecules that encode a spore surface protein (carrier) and the heterologous protein to be displayed (passenger); and (2) a non-recombinant approach based on spore adsorption, spontaneous interaction between negatively charged hydrophobic spores and purified proteins. Naturally, endospores offer unique resistance properties and can survive for long periods under a variety of stress conditions, such as high temperature, desiccation, lack of nutrients, and exposure to chemical solvents. These characteristics facilitate the storage and transport of these endospores. On the other hand, *Escherichia coli* enterotoxigenic (ETEC) are the major foodborne pathogens responsible for traveler's diarrhea. The main traits of virulence of this bacterium are the adhesins production and enterotoxins secretion. The treatment of diarrhea symptoms can involve, mainly, antibiotic therapy. However, data show resistance to antibiotics has increase worldwide. In this context, there is an urgent need for the development of new preventive strategies for the control of ETEC infections. Among them, a promising approach is the use of probiotics. The discovery of new virulence proteins and a better knowledge of the molecular events required for the effective delivery of toxins may provide additional way for the severe diarrhea control as well the development of effective treatment caused by these pathogens. The aim of this study was to investigate, using *in vitro* approaches, the inhibitory potential of *Bacillus* endospores against the human ETEC reference line H10407. We used the ETEC thermolabile toxin (LT) as a model to study the neutralization of toxin activity after adsorption by different endospores under different treatment conditions. Our results demonstrate a promising new probiotic activity of *Bacillus* endospores for the treatment of enteric diseases.

Keywords: probiotic, neutralization, toxin, bacillus, endospores.

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