

TITLE: IN VITRO EVALUATION OF THE BACTERICIDAL ACTIVITY OF BACILLUS SUBTILIS ON BACTERIA OF CLINICAL INTEREST IN VETERINARY MEDICINE

AUTORS: BARBOSA, F.B.¹; FRANCO, L.S.¹; CUNHA, M.P.V.¹; OLIVEIRA, M.G.X.¹; DAVIES, Y.M.¹; NISHIO, C.M.²; PICOVSKY, A.M.³; KNÖLB, T¹.

INSTITUTION: 1. FACULDADE DE MEDICINA VETERINÁRIA DA USP – FMVZ (RUA PROF. DR. ORLANDO MARQUES DE PAIVA, 87. CIDADE UNIVERSITÁRIA ARMANDO DE SALLES OLIVEIRA – CEP 05508-270 – SÃO PAULO - SP) – BRASIL; 2. NK SERVIÇOS DE CONSULTORIA LTDA (R. MAL. HASTIMPHILO DE MOURA, 338 APTO E23A, V. SUZANO, SÃO PAULO - SP, CEP 05641-000) – BRASIL; 3. BIOGENIC GROUP (RUA CÔNEGO EUGÊNIO LEITE, 1126, APTO 144 - CERQUEIRA CÉSAR - CEP 05414-001 - SÃO PAULO - SP) – BRASIL.

ABSTRACT

Due the emergence of bacterial resistance and restrictions on the use of antimicrobials as growth promoters in animal husbandry, new means of disease control are being developed, with incentive for the use of bacteria with a probiotic effect. *Bacillus subtilis*, gram-positive rhizobacterium, has been suggested as an alternative due the bacteriocins production capacity, volatile compounds and antifungal lipopeptides, able to act as a competitive agente in the occupation of sites in the intestinal mucosa. The scope of this study was to evaluate the *in vitro* inhibitory effect of *Bacillus subtilis* culture supernatant on bacteria of interest in veterinary medicine. The agent was cultured in LB broth (Luria Bertani), incubated at 37°C for 24 hours. The culture was standardized at 108 CFU/mL. After centrifugation and filtration, 100µL of the supernatant was added to a 96-well microplate containing 50µL of each test organism, standardized using McFarland for the 106 CFU/mL concentration. An optical density (OD) reading was performed on ELISA reader with filter 595 at time zero, with one more reading after 18 hours of incubation at 37°C. After the calculating difference between the final average OD and the initial average OD, the bactericidal activity (final OD < inicial OD) of the *Bacillus subtilis* culture supernatant was observed by reducing the of growth of the following agentes: *Salmonella* Heidelberg, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, Enteroaggregative *E. coli*, *Escherichia fergusonii*, *Proteus mirabilis* and *Staphylococcus intermedius*. The supernatant also inhibited the growth of yeast *Candida albicans*. For some agents, *Bacillus subtilis* culture supernatant didn't show *in vitro* efficacy (final OD > initial OD): *Pasteurella multocida*, *Listeria monocytogenes*, *Bordetella avium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Micrococcus luteus*. The data from this study suggests than *Bacillus subtilis* may be on option for antimicrobial in certain situations, although further studies are needed to demonstrate *in vitro* efficiency. It is important to consider that some pathogens haven't been eliminated by bacteriocins produced *in vitro* and there's a need to evaluate if the contínuos use of these probiotics in animal husbandry would not create selective pressure on the pathogens mentioned.

Keywords: *Bacillus subtilis*, bactericidal, resistance

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