

**TITLE:** Activity of cell free supernatants from *Bifidobacteria* cultures against pathogenic Gram negative bacilli

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**ABSTRACT:**

*Bifidobacteria* are dominant in the colon of healthy humans and are related to intestinal microbiota balance. The antagonistic effect of bifidobacteria on pathogenic microorganisms is directly related to the reduction of local pH by the production of organic acids, competition for nutrients, adhesion to mucosal cells, stimulation of cells of the host's immune system and production of bacteriocins. Some researchers suggest that the production of organic acids by *Bifidobacteria* is responsible for its inhibitory effect on bacteria, especially Gram negative bacilli. Based on that, the aim of this study were evaluate the antibacterial effect of three Cell Free Supernatants (CFSs) from *Bifidobacteria* cultures against Gram negative bacteria. CFSs were obtained from these cultures after centrifugation at 7.500 rpm, a 4°C por 10 min. The supernatants were filtered through a 0.22 µm cellulose membrane and with them, organic acids (lactic acid and acetic acid) and ethanol were quantified by gas chromatography with Flame Ionization Detector (GC-FID), followed by determination of their antibacterial activities against *Pseudomonas aeruginosa* (n=2), *Klebsiella pneumoniae* (n=2), *Escherichia coli* (n=2); *Salmonella* (n=2), *Shigella sonei* (n=1) *Vibrio cholerae* (n=1). GC-FID analysis of CFSs showed concentrations of lactic acid which ranged from 9.25 to 1,423.38 mM. For acetic acid and ethanol, these concentrations ranged from 276.90 to 174.66 mM and 26.45 to 40.19 mM respectively. CFSs were able to inhibit all pathogenic bacteria evaluated. The percentage of inhibition ranged from 68.5 to 89.0% depending on the bacteria evaluated. The antimicrobial activity was dependent on the CFSs tested. Based on these resultats, the presence of

organic acids and ethanol act synergistically by inhibiting Gram-negative bacilli.

**Keywords:** CFS, Bifidobacteria, GC-FID, Ethanol, organic acids

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