

Title: Effects of heat-inactivated *Lactobacillus rhamnosus* ATCC 7469 on macrophages challenged with *Staphylococcus aureus* and *Escherichia coli*.

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Abstract: Studies demonstrate that probiotic microorganisms, even non-viable, keep their beneficial effects. The objective of this study was to evaluate the effect of *L.rhamnosus* (ATCC 7469) inactivated in macrophage culture (RAW 264.7) challenged by *S.aureus* (ATCC 6538) and *E. Coli* (ATCC 25922), through the production of cytokines and nitric oxide (NO). Initially, suspension *L. rhamnosus* was carried out (5×10^7 cells/ml) which was sterilized (121 °C / 15 min), and after centrifugation, the *pellet* was resuspended in sterile and pyrogen-free physiological solution. Suspensions of *S. aureus* and *E. coli* (10^7 cells/ml) were also prepared. Macrophages were grown (37°C, 5% CO₂) for 2.5 h in six different situations: 1) presence only of *L. rhamnosus* heat inactivated; 2) inactivated *L. rhamnosus* and *S. aureus*; 3) inactivated *L. rhamnosus* and *E. coli*; 4) presence only of *S. aureus*; 5) presence only of *E. coli*; 6) and only with PBS (control group). Then the supernatants were removed, culture medium (DMEM) was added and macrophages were incubated further 16 h. Subsequently, the supernatants were collected and cytokine analysis performed by enzyme immunoassay ELISA and nitric oxide (NO) by Griess method. The results were compared and analyzed, using ANOVA and Tukey test, 5%. The results showed the cells cultivated with the bacterias, *S. aureus* or *E. coli*, and with only inactivated *L. rhamnosus* produced significant ($P < 0.05$) TNF- α , IL-6, IL-1 β , IL-12 and IL-10 and NO. On the experimental group challenged with *E. coli* there was greater production of cytokines than the control, in which the *L. ramnosus* was able to maintain (IL- 12, IL-6, TNF- α and IL-10) or lower (IL-1 β and NO) levels of the cytokines. In the group challenged with *S. aureus*, probiotic bacteria remained (TNF- α , IL-12) or greater stimulated (IL-1 β , IL-6, IL-10 and NO) compared to the control. *L. rhamnosus* alone was capable of inducing the production of TNF- α , IL-12 and NO greater than the control. In conclusion, *L. rhamnosus* inactivated, was able to modulate synthesis of different cytokines with pro-inflammatory functions (TNF- α , IL-6, IL-12, IL-1 β), or regulatory (IL-10) suggesting a role strain of *L. rhamnosus* ATCC 7469 heat inactivated in modulating or stimulating responses by macrophages.

Key-word: *Lactobacillus rhamnosus*, cytokines, macrophages, probiotics.