

Culture Supernatants from *Lactobacillus plantarum* Induce Necrosis on a Human Promyelocytic Leukemia Cell Line

M. A. Puertollano, E. Puertollano, G. Álvarez de Cienfuegos and M. A. de Pablo
Division of Microbiology, Department of Health Sciences, University of Jaén, Spain

Lactic acid bacteria (LAB) and bioactive peptides from these LAB are known to have antitumoral properties⁽¹⁾, although the intrinsic mechanisms responsible for the execution of this activity are poorly understood. Previous work in our laboratory reported that concentrated supernatants from *L. plantarum* reduce the development of HL-60 cells and increase the erythrocyte hemolysis⁽²⁾. Here we investigated the ability of concentrated supernatants from *L. plantarum* to promote cell death in this human promyelocytic cell line. This study was aimed to test the hypothesis that concentrated supernatants from *L. plantarum* at 5, 50 or 100 µg/ml for 24 h exert cytotoxic effects on HL-60 cells. Accumulation of reactive oxygen species (ROS) was diminished and nuclear staining with Hoechst 33342 and propidium iodide (PI) determined a necrotic induction in a concentration-dependent sequence. Concentrated supernatants did not modify or reduced the activity of caspase-3. The assessment of phosphatidylserine externalization by annexin V/PI double staining led to a necrotic state, but the treatment did not produce a dissipation of mitochondrial membrane potential ($\Delta\psi_m$), whereas cell cycle analysis revealed that concentrated supernatants failed to significantly enhance the population of HL-60 cells in the hypodiploid (sub-G1) fraction. In this study we demonstrated the cytotoxic properties of concentrated supernatants from *L. plantarum* on a tumour cell line, and then, to open the possibility to analyze the chemical composition to elucidate the bioactive molecules.

1. LeBlanc JG, Matar C, Valdez JC, LeBlanc J & Perdigon G (2002) *J Dairy Sci* 5, 2733–2742.

2. Puertollano E, Puertollano MA, Cruz-Chamorro L, Álvarez de Cienfuegos G, Ruiz-Bravo A & de Pablo MA (2009) *J Appl Microbiol* 106, 1194–1203.