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Effect of Bacillus pumilus ribonuclease on the paramagnetic centers of microbial cells

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Abstract

The potential clinical application of Bacillus pumilus cytotoxic ribonuclease (binase) for selectively inducing the death of tumor cells makes it imperative to investigate its effect on the normal human microflora. Flow cytometry was used to determine that binase concentration causing the apoptosis of cancer cells had no effect of the viability of Escherichia coli K12. The changes in the paramagnetic centers of E. coli K12 cells in the presence of nontoxic binase concentrations revealed by EPR spectroscopy included higher EPR signals from iron-containing proteins (including those from the Fe-S clusters) and of the Mn(II) hyperfine structure. The TMTH spin probe (N-(1-hydroxy-2,2,6,6-tetramethylpiperidine-4-il)-2-methylpropanamide hydrochloride) was used to reveal a twofold increase in the levels of reactive oxygen species (ROS) in the cells, which induced oxidative stress in the enzyme-treated bacteria. Inductively coupled plasma mass spectrometry revealed elevated contents of alkaline (Li, Na, K), alkali earth (Mg, Ca), transition (Cr, Mn, Fe, Cu, Zn), and post-transition metals (Bi, Pb) in the cells. Elevated levels of Cu and Zn (which impair the activity of the respiratory chain enzymes) and of Mn, which is known as a superoxide dismutase cofactor, confirmed development of the oxidative stress in bacteria. © 2013 Pleiades Publishing, Ltd.

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Keywords

Bacillus pumilus, binase, cytotoxic ribonucleases, EPR, intestinal microflora, oxidative stress, ROS, transition metals