

## Modulation of the synthesis of antimicrobial compounds in *Lactobacillus acidophilus* due to oxidative stress

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Limited knowledge exists on the effects of oxidative stress on probiotic bacteria, like *Lactobacillus*. Studies show varying impacts of oxidative stress on these bacteria, with implications for their growth and bactericidal activity. Understanding how oxidative stress influences the production of antimicrobial compounds is crucial. This research could have applications in the pharmaceutical industry by enhancing antibacterial metabolite synthesis without genetic manipulation.

The aim of this research is studying the oxidative stress impact induced by TiO<sub>2</sub> nanoparticles on the antibacterial capacity of *Lactobacillus acidophilus* ATCC 4756. To fulfill this aim the study of oxidative stress influence on probiotic bacteria growth and antibacterial activity of probiotics to planktonic and biofilm forms of pathogens were performed.

The growth of *L. acidophilus* ATCC 4756 was measured by spectrophotometry and CFU seeding. Antibacterial activity of probiotics was estimated by co-cultivation and agar wells methods.

Results showed that the optical density with increasing TiO<sub>2</sub> concentration in the range from 15 to 1000  $\mu\text{g/ml}$  varied from 0.2 to 0.5, with a control of 0.175. CFU at range of concentration 15-125  $\mu\text{g/ml}$  was more than control, only 250  $\mu\text{g/ml}$  equals the control -  $1 \cdot 10^8$  CFU/ml. Based on the inverse relationship between the CFU and the OD of the solution, it can be concluded about the possible effect of nanoparticles on the growth and metabolite synthesis of *Lactobacillus*.

Co-cultivation study of *Lactobacillus* + TiO<sub>2</sub> combination influence on planktonic pathogens showed that for *Escherichia coli* K12 TiO<sub>2</sub> decreases cell survival up to 56% at a concentration of 1 mg/ml. With a joint culture of *Lactobacillus* and TiO<sub>2</sub>, the survival rate decreased to 25.7% at the same concentration. For *Staphylococcus aureus*, similar values were 72.5% and 19.7%, respectively.

Significant effects were observed in a co-cultivation experiment with *E. coli* K12 and *S. aureus* biofilms, showing inhibition zones when *Lactobacillus* + TiO<sub>2</sub> were used. No inhibition zones were present in the control group. *Lactobacillus* + TiO<sub>2</sub> in concentrations of 15, 250, and 500  $\mu\text{g/ml}$  resulted in varying inhibition zone diameters  $1.12 \pm 0,04$  cm,  $1.13 \pm 0,05$  cm,  $1.2 \pm 0,03$  cm cm respectively.

This study emphasizes the positive effect of oxidative stress on producing antibacterial compounds in *Lactobacillus*. Future research will aim to understand the mechanisms involved in this process. The research also validates the use of TiO<sub>2</sub> to boost the production of antimicrobial compounds in probiotic bacteria against pathogens.