Fourier transform infrared spectroscopy as a tool to evaluate the effect of Lactobacillus plantarum supernatant on Pseudomonas aeruginosa biofilm development

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Chronic wounds, by definition, are wounds that remain in a chronic inflammatory state and therefore fail to follow normal patterns of the healing process. They represent a significant burden to patients and health care professionals because they are very difficult to heal, inflict a huge cost to society and impair the quality of life for millions of people. Infection is a common problem in chronic wounds. Among hospitalized patients, 8–10% is susceptible to infection by opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa* which are notorious for forming chronic, biofilm-based infections in their hosts.

Treatment of infection in chronic wounds represents a significant challenge. The use of harmless bacteria to displace pathogenic organisms is an alternative and promising way of combating infections. Using good bacteria to obstruct bad ones - a strategy known as bacterial interference - is one application of so-called probiotics.

The aim of the present work was to study the interference and / or inhibition of *Lactobacillus plantarum* supernatant on *P. aeruginosa* biofilms by using Fourier transform infrared spectroscopy and others cooperative techniques. For this purpose we analyzed the effect of *L. plantarum* supernatants and neutralized *L. plantarum* supernatants, on biomass accumulation, quorum sensing signal molecules, and virulence factors such as elastase, rhamnolipid and pyocyanin of *P. aeruginosa* growing as biofilm.

Biofilm were produced either in column bioreactors packed with polypropylene beads (when high amounts of sessile cells were required) or in continuous-flow chambers using borosilicate slides, ZnSe windows and silicone tubes as abiotic surfaces for growth. Biofilm biomass was quantified by crystal violet stain and the viability of sessile cells was evaluated by Syto 9-propidium iodide stain. Biomass accumulation and the biofilm chemical composition were monitored by FT-IR spectroscopy. Quorum sensing signals were studied by FT-IR, GCMS, and TLC using *Agrobacterium tumefaciens* KYC55 as biosensor. Virulence factors production was evaluated by: i) elastase by Congo red-elastin technique, ii) rhamnolipids by hemolysis of sheep blood and inhibition of *Bacillus subtilis* growth, and iii) pyocyanin by extraction and reading at DO_{520}.

An inhibition effect of around 70% on biomass accumulation was observed. In addition, an effect on quorum sensing signals and virulence factors production was also detected.

This study provides clear evidences that FT-IR spectroscopy can be used to monitor the effect of *L. plantarum* supernatant on *P. aeruginosa* biofilm.