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Mechanism of antibacterial action of a citrus essential oil on *Escherichia coli* and *Lactobacillus rhamnosus*

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The banning of antibiotic use in animal feed since 2006 by European Community, due to the concern about the emergence and spreading of bacterial resistance to antibiotics, has led to the search of alternatives to substitute them. Currently, essential oils (EOs) have received great attention as possible alternative due to their antimicrobial properties. Specifically the citrus EOs, that are by-products of orange juice production, could be an excellent alternative for this purpose since they have shown to have a good potential to fight pathogenic bacteria (Fisher and Phillips, 2008) and their use in animal feed could become feasible since there is a high availability of these oils in the worldwide market . Therefore, the aim of this study was to evaluate the mechanism of antibacterial action of a *Citrus* EO, Brazilian orange terpens (BOT), on a pathogenic bacterium and a beneficial bacterium that can occur in pig gut microbiota. The pathogenic bacterium was *E. coli* U21 (K88 LT/STb/F18/STa) isolated from pig gut and the beneficial bacterium was *L. rhamnosus* ATCC 7469. Firstly, Minimal Inhibitory Concentration (MIC) was determined by microdilution method and Minimal Bactericidal Concentration (MBC) by plating. MICs and MBCs results showed that BOT was more active on *E. coli* than *L. rhamnosus*, since MICs and MBCs lower observed to the pathogenic bacterium. Then, the effect of this EO on the permeability of bacterial cell membrane was evaluated by electrical conductivity measurements, the effect on the integrity of cell membrane by measuring the release of cell constituents (proteins and sugar reducers), and the morphological changes on bacterial cells were observed by scanning electron microscopy (SEM). The results showed that BOT produced a higher increase on the permeability of cell membrane of *E. coli* than *L. rhamnosus* as the EO concentration and the time of exposure were increased (1, 3, 6 and 9h). The release of cell constituents, proteins and reducing sugars were higher as the EO concentration was increased. SEM analysis ratified higher disturbances and damages on the normal morphology of the pathogenic bacterium than of the beneficial bacterium. Therefore, these results highlight the selective antibacterial activity of this citrus EO, having higher effect on the pathogenic bacterium and being its mechanism of antibacterial action, the increase of permeability associated to disrupting effects in the integrity of bacterial cell membrane.

1. Fisher, K. and Phillips, C. (2008) 'Potential antimicrobial uses of essential oils in food: is citrus the answer?', Trends in Food Science and Technology, 19(3), pp. 156–164.

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