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Exploratory Tests of Crude Bacteriocins from Autochthonous Lactic Acid Bacteria against Food-Borne Pathogens and Spoilage Bacteria

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Abstract : The aim of the present work was to test in vitro inhibition of food pathogens and spoilage bacteria by crude bacteriocins from autochthonous lactic acid bacteria. Thirty autochthonous lactic acid bacteria isolated previously, belonging to the genera: Lactobacillus, Carnobacterium, Lactococcus, Vagococcus, Streptococcus, and Pediococcus, have been screened by an agar spot test and a well diffusion assay against Gram-positive and Gram-negative harmful bacteria: Bacillus cereus, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 8739, Salmonella typhimurium ATCC 14028, Staphylococcus aureus ATCC 6538, and Pseudomonas aeruginosa under conditions means to reduce lactic acid and hydrogen peroxide effect to select bacteria with high bacteriocinogenic potential. Furthermore, crude bacteriocins semiquantification and heat sensitivity to different temperatures (80, 95, 110°C, and 121°C) were performed. Another exploratory test concerning the response of St. aureus ATCC 6538 to the presence of crude bacteriocins was realized. It has been observed by the agar spot test that fifteen candidates were active toward Gram-positive targets strains. The secondary screening demonstrated an antagonistic activity oriented only against St. aureus ATCC 6538, leading to the selection of five isolates: Lm14, Lm21, Lm23, Lm24, and Lm25 with a larger inhibition zone compared to the others. The ANOVA statistical analysis reveals a small variation of repeatability: Lm21: 0.56%, Lm23: 0%, Lm25: 1.67%, Lm14: 1.88%, Lm24: 2.14%. Conversely, slight variation was reported in terms of inhibition diameters: 9.58± 0.40, 9.83± 0.46, and 10.16± 0.24 8.5 ± 0.40 10 mm for, Lm21, Lm23, Lm25, Lm14and Lm24, indicating that the observed potential showed a heterogeneous distribution (BMS = 0.383, WMS = 0.117). The repeatability coefficient calculated displayed 7.35%. As for the bacteriocins semiquantification, the five samples exhibited production amounts about 4.16 for Lm21, Lm23, Lm25 and 2.08 AU/ml for Lm14, Lm24. Concerning the sensitivity the crude bacteriocins were fully insensitive to heat inactivation, until 121°C, they preserved the same inhibition diameter. As to, kinetic of growth , the µmax showed reductions in pathogens load for Lm21, Lm23, Lm25, Lm14, Lm24 of about 42.92%, 84.12%, 88.55%, 54.95%, 29.97% in the second trails. Inversely, this pathogen growth after five hours displayed differences of 79.45%, 12.64%, 11.82%, 87.88%, 85.66% in the second trails, compared to the control. This study showed potential inhibition to the growth of this food pathogen, suggesting the possibility to improve the hygienic food quality.

Keywords : exploratory test, lactic acid bacteria, crude bacteriocins, spoilage, pathogens
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