

**Title** Biopreservation of fresh-cut salads using bacteriocinogenic lactic acid bacteria isolated from commercial produce

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### Abstract

The food industry is seeking novel biopreservatives such as active lactic acid bacteria (LAB) cultures and/or their bacteriocins to control psychrotrophic pathogens including *Listeria monocytogenes*. However, most bacteriocins have been extracted from and applied to meat and dairy products with little emphasis on fresh produce. The objective of this study was to isolate and characterize bacteriocinogenic LAB strains of plant origin for applications on fresh-cut fruit and vegetable products.

Fourteen different vegetable products were investigated for content of native LAB. From 218 potential LAB isolates, 92 were confirmed catalase negative and gram positive. The antimicrobial properties of cell-free supernatants from these isolates were screened using the agar diffusion bioassay with *Lactobacillus sakei* and *Listeria innocua* as indicator organisms. Eight strains isolated from mung bean sprouts, swiss chard and mini-seedless cucumbers inhibited both indicator organisms. The presence of bacteriocin-like substances (BLS) was indicated by inhibition zones following the neutralization of pH and elimination of H<sub>2</sub> O<sub>2</sub>. These zones were lost following the addition of proteolytic enzymes. The eight LAB isolates were identified using 16S rRNA gene sequencing as *Lactococcus lactis* ssp. *lactis* (1) and *Enterococcus faecium* (7). Antimicrobial effectiveness was tested against common fresh-cut produce pathogens and spoilage organisms at 5 and 20 °C. The BLS showed significant antimicrobial effects against *Li. innocua* , with a mean inhibition zone diameter of 9.7 mm at 5 °C and 10.2 mm in diameter at 20 °C. However, only the organic acids and not the H<sub>2</sub> O<sub>2</sub> or BLS showed strong antimicrobial effects against *Pseudomonas fluorescens*, *Erwinia carotovora*, *Bacillus cereus* and weak effects against *Leuconostoc mesenteroides* . A further investigation of the eight BLS producing LAB against *Penicillium expansum*, *Botrytis cinerea* and *Monilinia fructicola* spores using the microtiter plate method showed that inhibition was due to mainly to the production of organic acids and partially due to H<sub>2</sub> O<sub>2</sub> not the BLS.

The effects of both *L. lactis* and *Ent. faecium* on the natural microflora and on *Listeria* inoculated fresh-cut salads were investigated. Bacteriocinogenic LAB applied to fresh-cut salad inoculated with *Li. innocua* reduced *Listeria* counts significantly ( $p=0.005$ ) when compared to the control plates with LAB,

during a 10 day storage trial at 5 °C. The addition of LAB was able to significantly reduce the counts of natural *Listeria* sp. ( $p=0.033$ ), yeasts ( $p=0.011$ ), *Pseudomonas* sp. ( $p=0.010$ ) and total conforms ( $p=0.011$ ) in comparison to the controls. Overall, results suggest that *L. lactis* and *Ent. faecium* could be used as a successful biopreservative because they were effective at controlling both spoilage and pathogenic organisms when applied as protective cultures to fresh-cut salad during cold-storage, thereby improving the product shelf life and safety.