

Identification of the Antimicrobial Effect of Liquorice Extracts on Gram-Positive Bacteria: Determination of Minimum Inhibitory Concentration and Mechanism of Action Using a luxABCDE Reporter Strain

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Abstract : Natural preservatives have been used as alternatives to traditional chemical preservatives; however, a limited number have been commercially developed and many remain to be investigated as sources of safer and effective antimicrobials. In this study, we have been investigating the antimicrobial activity of an extract of *Glycyrrhiza glabra* (liquorice) that was provided as a waste material from the production of liquorice flavourings for the food industry, and to investigate if this retained the expected antimicrobial activity so it could be used as a natural preservative. Antibacterial activity of liquorice extract was screened for evidence of growth inhibition against eight species of Gram-negative and Gram-positive bacteria, including *Listeria monocytogenes*, *Listeria innocua*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*. The Gram-negative bacteria tested include *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium* but none of these were affected by the extract. In contrast, for all of the Gram-positive bacteria tested, growth was inhibited as monitored using optical density. However parallel studies using viable count indicated that the cells were not killed meaning that the extract was bacteriostatic rather than bacteriocidal. The Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal Concentration [MBC] of the extract was also determined and a concentration of 50 $\mu\text{g ml}^{-1}$ was found to have a strong bacteriostatic effect on Gram-positive bacteria. Microscopic analysis indicated that there were changes in cell shape suggesting the cell wall was affected. In addition, the use of a reporter strain of *Listeria* transformed with the bioluminescence genes *luxABCDE* indicated that cell energy levels were reduced when treated with either 12.5 or 50 $\mu\text{g ml}^{-1}$ of the extract, with the reduction in light output being proportional to the concentration of the extract used. Together these results suggest that the extract is inhibiting the growth of Gram-positive bacteria only by damaging the cell wall and/or membrane.

Keywords : antibacterial activity, bioluminescence, *Glycyrrhiza glabra*, natural preservative

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