FORMATION AND CONTROL OF BIOFILMS OF THERMO-RESISTANT STREPTOCOCCI ON STAINLESS STEEL

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ABSTRACT

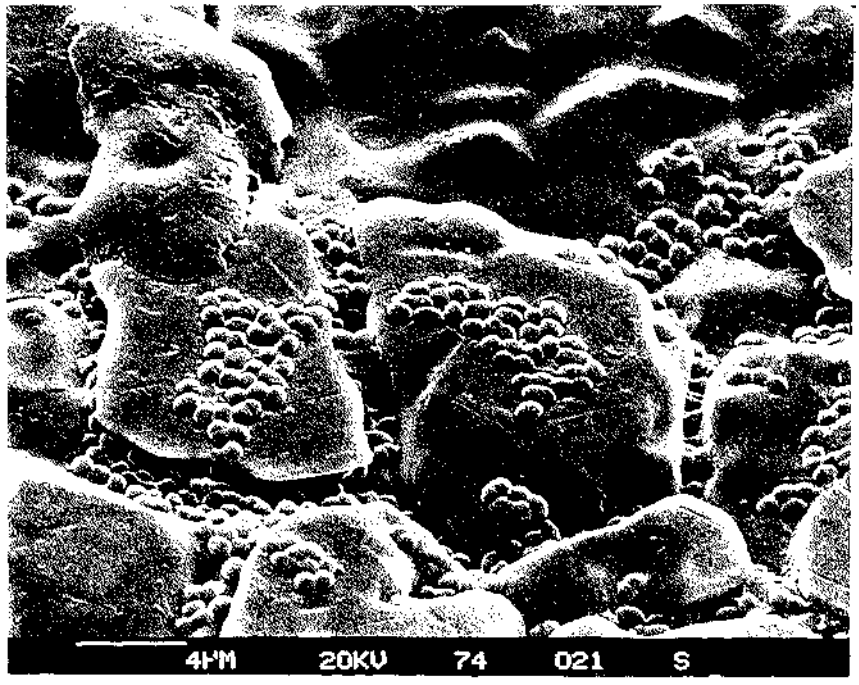
The aim of this study was to develop improved methods of controlling biofilms of thermo-resistant streptococci in dairy manufacturing plant.

A method to rapidly and accurately detect viable cells of thermo-resistant streptococci on stainless steel surfaces involving the use of the Malthus microbiological growth analyser was developed. A modified Robbins device was designed and installed in a dairy manufacturing plant to monitor biofilm development and obtain isolates for study. These studies confirmed that routine cleaning programmes were not eliminating biofilms of thermo-resistant streptococci from the stainless steel surface. The isolates obtained were identified using biochemical and molecular techniques. As well as the expected *Streptococcus thermophilus,* a new species, *S. waiu* representing 24% of the isolates was also described. Molecular techniques (polymerase chain reaction and fluorescent *in situ* hybridisation) were developed to rapidly identify the bacteria. The cell surface hydrophobicity of all isolates was determined, with those obtained from dairy manufacture being highly hydrophobic compared with mixed hydrophobicity in the general population.

There was no correlation between many factors often associated with adhesion (such as hydrophobicity, polysaccharide production, surface charge) and the rate of cell adhesion. However, treatment of the bacteria with proteolytic agents reduced the number of all isolates adhering to stainless steel by approximately 100-fold. A 55 kDa protein with an N-terminal sequence matching that of β-lactoglobulin was identified as being associated with adhesion, through comparisons between cell proteins separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis

before and after treatment with proteolytic agents. Further evidence of the involvement of this protein in adhesion was the reduction in adhesion following treatment of the cells with specific antiserum to the 55 kDa "adhesion protein". The presence of the protein on the surface of the cells was demonstrated by immunolabelling.

A continuous flow laboratory reactor was developed to generate biofilms of thermo-resistant streptococci on stainless steel surfaces in the presence of skim milk. Trials using biofilms developed in laboratory reactors and on the surface of coupons in pilot plants, indicated that chemicals routinely used in dairy manufacturing plants were inadequate to remove or inactivate thermo-resisant streptococci. Proteolytic enzyme treatments removed more bacteria from the surface than any other treatment, reducing the total number of cells by at least 100-fold. This was confirmed in a pilot-scale trial using a commercial proteolytic-enzyme-based cleaner. In addition, no viable cells were detected following treatment with this cleaner. Proteolytic enzyme cleaners may be more effective than the caustic and acid cleaners for the routine cleaning of biofilms of thermo-resistant streptococci from dairy manufacturing plants.



Frontispiece Scanning electron micrograph of *Streptococcus thermophilus* colonising 316 stainless steel with a 2b surface finish as used in dairy manufacturing plants. The cells are in clumps rather than as individual cells and appear to be preferentially colonising the interfaces between the oxide grain boundaries on the surface of this stainless steel sample.
Magnification = 3600 x.

LIST OF PUBLICATIONS

This work has been published in part in the following papers:

1. Flint, S. H., Bremer, P. J., Brooks, J. D. (1997). Biofilms in dairy manufacturing plant - description, current concerns and methods of control. *Biofouling,* **11,** 81-97.

2. Flint, S. H., Brooks, J. D., Bremer, P. J. (1997). Use of the Malthus conductance growth analyser to monitor biofilms of thermophilic streptococci on stainless steel. *Journal of Applied Microbiology,* **83,** 335-339.

3. Flint, S. H., Brooks, J. D., Bremer, P. J. (1997). The influence of cell surface properties of thermophilic streptococci on attachment to stainless steel. *Journal of Applied Microbiology,* **83,** 508-517.

4. Flint, S. H., Brooks, J. D., van den Elzen, H., Bremer, P. J. (1997). Biofilms in dairy manufacturing plant - a threat to product quality. *The Food Technologist*, **27,** 61-64.

5. Flint, S. H., Ward, L. J. H., Brooks, J. D. (1998). Characterization of a thermophilic streptococcus from a biofilm, description of *Streptococcus waiu* sp. nov. and development of a species-specific fluorescent probe. *International Journal of Systematic Bacteriology*, (Submitted).

LIST OF PRESENTATIONS

This work has been presented in part in the following presentations at scientific conferences:

1. Flint, S. H., Scholts, W., Kells, N. J., Wiles, P. J. (1996). Evaluation of the development of biofilms on different welded surface. *International Pacific Welding Conference,* Auckland, February 1996.

2. Flint, S. H., Bremer, P. J., Brooks, J. D. (1996). The influence of topography on the development of biofilms of thermophilic streptococci on stainless steel surfaces. *New Zealand Microbiological Society Conference*, Christchurch, October 1996.

3. Flint, S. H., Brooks, J. D., Bremer, P. J. (1996). Factors affecting the attachment of *Streptococcus bovis* and *Bacillus cereus* to stainless steel surfaces. *New Zealand Microbiological Society Conference,* Christchurch, October 1996.

4. Flint, S. H., Brooks, J. D., Bremer, P. J. (1996). The influence of cell surface properties of thermophilic streptococci on attachment to stainless steel. *American Society for Microbiology Conference on Microbial Biofilms*, Utah, USA, October 1996.

5. Flint, S. H., Brooks, J. D., van den Elzen, H., Bremer, P. J. (1997). Biofilms in dairy manufacturing plant - a threat to product quality. *New Zealand Institute of Food Science and Technology Conference,* Napier, July 1997.

   Winner of the Bronson and Jacobs Award for Excellence in Food Science and Technology - best paper presented at the conference.

6. Flint, S. H., Brooks, J. D., Bremer, P. J. (1997). The influence of cell surface properties of thermophilic streptococci on attachment to stainless steel. *New Zealand Institute of Food Science and Technology Conference,* Napier, July 1997.

7. Flint, S. H., Bremer, P. J., Brooks, J. D. (1997). The influence of topography on the development of biofilms of thermophilic streptococci on stainless steel surfaces. *New Zealand Institute of Food Science and Technology Conference,* Napier, July 1997.

8. Flint, S. H., Ward, L. J. H., Brooks, J. D., Bremer, P. J. (1997). Description of *Streptococcus waiu* sp. nov. *New Zealand Microbiological Society Conference,* Rotorua, November 1997.

9. Flint, S. H., Brooks, J. D., Bremer, P. J. (1997). Adhesion mechanisms of thermophilic streptococci. *New Zealand Microbiological Society Conference,* Rotorua, November 1997.

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