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Surface characteristics of an adhesive thermophilic spore-forming *Bacillus*, isolated from milk powder

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Food Technology

At Massey University, Palmerston North, New Zealand

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ABSTRACT

The growth of thermophiles during the manufacture of milk powder leads to a progressive increase in the number of thermophilic bacteria contaminating the final product. The limited residence time of the milk in the plant during milk powder manufacture and the concentration effect of converting milk into milk powder cannot explain the number of thermophiles found in the final product. This suggests that thermophiles are attaching to the large surface area of stainless steel found within a milk powder plant and then growing and developing into biofilms, with individual cells and/or biofilm fragments sloughing off into the product line and thus contaminating the final product.

The aim of the present study was to investigate the attachment mechanisms that enable the thermophile *Anoxybacillus flavithermus* (B12) to attach to stainless steel surfaces. Passing a B12 culture through a column of stainless steel chips, collecting the first cells to pass through, re-culturing and repeating the process six times, resulted in the isolation of a mutant, labelled X7, with 10-fold reduced ability to attach to stainless steel as well as a reduced ability to attach to plastic and glass.

A comparison of bacterial cell surface properties indicated that X7 was less hydrophobic than its parental strain B12. Cell surface charge measurements also suggest that X7 has less net negative surface charge. Disruption of extracellular polysaccharides and DNA appeared to have no effect on the attachment process. Removal of surface proteins caused a reduction in attachment of B12 and X7 as well as a reduction in surface hydrophobicity suggesting surface protein involvement in both.

Analysis by two-dimensional gel electrophoresis of lysozyme/mutanolysin extracted surface proteins revealed two proteins expressed at reduced levels in X7 compared with B12. One protein was identified by mass spectrometry as the cytoplasmic enzyme Formate acetyltransferase. The role of Formate acetyltransferase and the
second unidentified protein on the attachment process of *Anoxybacillus flavithermus* remains unclear.
LIST OF PUBLICATIONS


LIST OF PRESENTATIONS


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</thead>
<tbody>
<tr>
<td>g</td>
<td>acceleration due to gravity</td>
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<tr>
<td>AFM</td>
<td>atomic force microscope</td>
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### Amino acids

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### Nucleotides

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