# Effect of cations on biofilm formation by *Geobacillus* species and *Anoxybacillus flavithermus* dairy isolates

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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2013

# Abstract

The concentration of free cations is one factor that may influence biofilm formation and consequent contamination of milk formulations by *Geobacillus* spp. and *Anoxybacillus flavithermus* during the manufacture of milk powders.

Culture optical densities were measured to show that $Ca^{2+}$ and $Mg^{2+}$ predominantly increased the planktonic growth of *Geobacillus* spp. and *A. flavithermus* cultures. Culture cell numbers were enumerated, and a protein quantification assay was used to indicate that increases in optical density elicited by $Ca^{2+}$ and $Mg^{2+}$ supplementation was due to increased production of bacterial surface protein rather than an increase in cell numbers.

High individual concentrations of $Na^{+}$, $K^{+}$ or $Ca^{2+}$ (63 – 250 mM) inhibited the planktonic growth of *Geobacillus* spp., and $Mg^{2+}$ protected *Geobacillus* spp. from high, inhibitory concentrations of $Na^{+}$, $K^{+}$ or $Ca^{2+}$.

The number of viable cells attached to stainless steel coupons was enumerated to show that cation concentrations or the monovalent to divalent cation ratio (2:1 compared to 10:1) did not influence the transition of bacteria from a planktonic to surface-attached form, or the subsequent formation of an established biofilm. However, preconditioning of the bacteria with cations increased their subsequent attachment. It was proposed that the transition of bacteria from a planktonic to surface-attached form is primarily mediated by the expression of bacterial surface proteins, as induced by cation preconditioning.

The number of attached *Geobacillus* spp. was up to 4 log CFU $cm^{-2}$ lower, for up to 18 h of biofilm formation, in a milk formulation that had a high monovalent to divalent cation ratio (greater than 10:1) relative to a milk formulation that had a monovalent to divalent cation ratio that resembled that found in unprocessed milk. Supplementation of a milk formulation that had a high monovalent to divalent cation ratio with $Ca^{2+}$ or $Mg^{2+}$ fully alleviated the inhibitory effect of the milk formulation on biofilm formation by *Geobacillus* spp.

It was concluded that there is potential for the total thermophile count in milk powders that have high monovalent to divalent cation ratios to be markedly reduced. This would increase the quality and selling price of the milk powders.

# Acknowledgements

I would like to thank my supervisors for their support and guidance. Thanks to Steve Flint for creating a very supportive environment, having a positive and enthusiastic attitude, and sharing insightful ideas – you were the glue in the matrix. Thanks to Denise Lindsay for putting a huge amount of time and effort into this project - particularly with editing manuscripts, your mentoring and excellent help with experimental design. Thanks to Jon Palmer for providing great support with the molecular biology aspects of the project and providing a different point of view. Thanks to John Brooks for your meticulous editing skills and support in experimental design and troubleshooting – and being the life of the party at the ASM Biofilms conference in Miami. Thanks to Edward Smolinski for your passion and sharing the observations and theories that triggered the start of this project.

Thanks to the Ministry of Science and Innovation and the Fonterra Co-operative Group for providing financial support for this project.

Thanks to Steve Holroyd and the Analytical Development team, and Kevin Palfreyman, at the Fonterra Research and Development Centre for backing this project and providing the opportunity to kickstart my career in Microbiology.

Thanks to my parents for encouraging me to do a PhD and allowing me to overstay at their place. Thanks to my girlfriend, Jessica Childs, for the great deal of assistance and understanding, and for knowing what it takes to finish post-graduate study.

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# List of publications

## Published manuscripts

**Somerton, B., Palmer, J., Brooks, J., Smolinski, E., Lindsay D. & Flint, S. (2012).** Influence of cations on growth of thermophilic *Geobacillus* spp. and *Anoxybacillus flavithermus* in planktonic culture. *Appl Environ Microbiol* **78**, 2477-2481.

**Somerton, B., Palmer, J., Brooks, J., Flint, S. & Lindsay, D. (2013).** Preconditioning with cations increases the attachment of *Anoxybacillus flavithermus* and *Geobacillus* species to stainless steel. *Appl Environ Microbiol* **79**, 4186-4190.

# List of conference presentations

## Oral presentations

**Somerton, B., Palmer, J., Brooks, J., Smolinski, E., Lindsay, D. & Flint, S. (2011).** Influence of cations on growth of thermophilic *Geobacillus* spp. and *Anoxybacillus flavithermus* in planktonic culture. 57th Annual Scientific Meeting of the New Zealand Microbiological Society Conference 2011 (Palmerston North, New Zealand).

**Somerton, B., Palmer, J., Brooks, J., Smolinski, E., Lindsay, D. & Flint, S. (2012).** High sodium and low calcium and magnesium concentrations in milk collectively inhibit biofilm formation by *Geobacillus* spp. 58th Annual Scientific Meeting of the New Zealand Microbiological Society Conference 2012 (Dunedin, New Zealand).

## Poster presentations

**Somerton, B., Palmer, J., Brooks, J., Smolinski, E., Flint, S. & Lindsay, D. (2011).** Physico-chemical and physiological role that cations play on attachment and early biofilm formation by *Anoxybacillus flavithermus*. 57th Annual Scientific Meeting of the New Zealand Microbiological Society Conference 2011 (Palmerston North, New Zealand).

**Somerton, B., Palmer, J., Brooks, J., Smolinski, E., Flint, S. & Lindsay, D. (2012).** Preconditioning of *Geobacillus* sp. and *Anoxybacillus flavithermus* with cations increases their attachment to stainless steel. 6th American Society for Microbiology Conference on Biofilms 2012 (Miami, USA).

| | |
|---|---|
| *g* | acceleration due to gravity |
| ATP | adenosine triphosphate |
| α | alpha subunit |
| $NH_3^+$ | amine ion |
| * | asterisk |
| bp | base pair(s) |
| β | beta subunit |
| Bap | biofilm-associated protein |
| $Cd^{2+}$ | cadmium ion |
| $CaCl_2$ | calcium chloride |
| $Ca^{2+}$ | calcium ion |
| CM 1:5 | calcium ion to magnesium ion ratio of 1:5 |
| $Ca_3(PO_4)_2$ | calcium phosphate |
| CWM | cell wall material |
| PS-CWM | cell wall material stripped of phosphate groups |
| CM-CWM | cell wall material with masked carboxylate groups |
| cm | centimetre |
| $Citr^{3-}$ | citrate ion |
| $Co^{2+}$ | cobalt ion |
| CFU | colony forming unit(s) |
| C | cytosine |
| Da | daltons |
| °C | degrees celcius |
| DNA | deoxyribonucleic acid |

| | |
|---|---|
| rDNA | deoxyribonucleic acid that encodes for a ribosomal gene |
| DLVO | Derjaguin, Landau, Verway, Overbeek |
| $H_2O$ | dihydrogen oxide |
| $H_2PO$ | dihydrogen phosphate ion |
| D | D orientation of an isomer |
| DSM | DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) bacteria collection reference number |
| EF | E and F helixes of a protein joined by a loop |
| EGTA | ethylene glycol-bis(β-aminoethyl ether)-N,N-tetraacetic acid |
| EPS | extracellular polymeric substances |
| $Fe^{2+}$ | iron (II) ion |
| Gy | Grays |
| G | guanosine |
| His | histidine |
| h | hour(s) |
| $HCitr^{2-}$ | hydrogen citrate ion |
| $H^+$ | hydrogen ion |
| $HPO_4^{2-}$ | hydrogen phosphate ion |
| κ | kappa |
| kg | kilogram |
| $Pb^{2+}$ | lead ion |
| Log | logarithm |
| L | L orientation of an isomer |
| $MgCl_2$ | magnesium chloride |
| $Mg^{2+}$ | magnesium ion |
| $Mn^{2+}$ | manganese ion |

| | |
|---|---|
| MS | mass spectroscopy |
| m/z | mass to charge ratio |
| MALDI-TOF | matrix assisted laser desorption/ionization-time of flight |
| µl | microliter |
| µmol | micromole |
| µM | micromole per liter |
| MF | milk formulation(s) |
| MPCA | milk plate count agar |
| mg | milligram |
| mm | millimeter |
| mmol | millimole |
| mM | millimole per liter |
| mV | millivolts |
| min | minute |
| mins | minutes |
| MDCR | monovalent to divalent cation ratio |
| X | multiplication factor |
| nm | nanometer |
| nM | nanomole per liter |
| $Ni^{2+}$ | nickel ion |
| N/A | not applicable |
| *n* | number of replicates |
| σ | omega (symbol for the population standard deviation) |
| PCR | polymerase chain reaction |
| KCl | potassium chloride |
| $K^{+}$ | potassium ion |

| | |
|---|---|
| pH | power of hydrogen |
| *P* | probability of detection |
| Pty Ltd | Proprietary Limited |
| rpm | revolutions per minute |
| 16S | ribosomal subunit number 16 |
| s | second(s) |
| NaCl | sodium chloride |
| $Na^+$ | sodium ion |
| NKC | sodium ion to potassium ion to calcium ion ratio of 1:1:2 |
| S-layer | Slime layer |
| spp. | species (plural) |
| sp. | species (single) |
| SD | standard deviation |
| SAS | statistical analysis software |
| s | subunit |
| TVC | total viable cells |
| $Zn^{2+}$ | zinc ion |

# List of tables