Enzymes produced by bacteria within biofilms of dairy origin and their effect on dairy products

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ABSTRACT

Prior to the current study, there was no scientific evidence that enzymes produced by bacteria within biofilms in milk transport tanker could have a detrimental effect on the quality of dairy products.

Bacteria attached to the internal surfaces of milk tankers were isolated, identified, and characterized in terms of their ability to produce heat-stable enzymes (protease and lipase) and to form biofilms. Twelve of the bacterial isolates were identified by 16s DNA sequencing as belonging to the genera *Bacillus, Staphylococcus*, *Streptococcus*, *Pseudomonas*, and *Serratia.*

Six of the dairy bacterial isolates were evaluated for their ability to produce proteolysis in milk when growing within either single culture or co-culture biofilms in an *in vitro* model system that simulated the upper part of a milk tanker during a typical summer's day of milk collection in New Zealand. Proteolysis per cfu decreased as the temperature of incubation increased (20−37 °C), and proteolysis per cfu was generally higher within biofilms compared with the corresponding planktonic cultures.

Lipolysis by bacteria within biofilms in the in *vitro* model was investigated using single or co-culture biofilms or planktonic cultures of four dairy bacteria and a known lipase-producing bacterium. The hydrolysis of *p*-nitrophenol palmitate was at least 10 times higher by bacteria within biofilms (0.01 to 8.35 nU/CFU) than in planktonic cultures (0.01 to 0.07 nU/CFU).

The effect of proteases on UHT skim milk was determined by exposing sterile skim milk to a multispecies biofilm formed on an *in vitro* model of a milk tanker. The amount of free peptides which indicated proteolysis in the UHT milk was monitored over five months of storage. Free peptides were higher in UHT milk that had been made from milk exposed to the multispecies biofilm, than in UHT milk that had been made from milk that had not been exposed to the biofilm. Enzymes that are secreted from biofilms into raw milk during transportation can potentially reduce the quality of dairy products. Improvements at this early stage of dairy manufacture may reduce economic loss in the dairy industry.

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Published Manuscripts

1. Teh, K. H., Flint, S., Palmer, J., Lindsay, D., Andrewes, P., Bremer, P., 2011. Thermo-resistant enzyme-producing bacteria isolated from the internal surfaces of raw milk tankers. *International Dairy Journal* 21, 742−747.

2. Teh, K. H., Flint, S., Palmer, J., Andrewes, P., Bremer, P., Lindsay, D., 2012. Proteolysis produced within biofilms of bacterial isolates from raw milk tankers. *International Journal of Food Microbiology* 157, 28-34.

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1. Teh, K. H., Lindsay, D., Palmer, J., Andrewes, P., Bremer., P., Flint, S., 2012. Proteolysis in ultra-heat-treated skim milk after exposure to multispecies biofilms under conditions modeling a milk tanker. *International Journal of Dairy Technology.*

LIST OF CONFERENCE PRESENTATIONS

Oral Presentations

1. Teh, K. H., Flint, S., Palmer, J., Lindsay, D., Andrewes, P., Bremer, P. (2011). The occurrence of thermo-resistant enzyme-producing bacteria isolated from raw milk tanker surfaces. 57th Annual Scientific Meeting of the New Zealand Microbiological Society Conference 2011 (Palmerston North, New Zealand).

2. Teh, K. H. (2012). Biofilms – Bacteria on the internal surface of milk tankers. New Zealand Large Herd Association Inc (NZLHA) conference 2012 (Palmerston North, New Zealand)

3. Teh, K. H., Flint, S., Palmer, J., Lindsay, D., Andrewes, P., Bremer, P. (2012). Increased lipolysis within dairy biofilms cells. The New Zealand Institute for Food Science and Technology Inc (NZIFST) Conference 2012 (Hamilton, New Zealand).

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ABBREVIATIONS

4-MU	4-methylumbelliferone
AHL	*N*-acylhomoserine lactone
BNA	Biofilm formation on the stainless steel surface under nutrient abundance
BNL	Biofilm formation on the stainless steel surface under nutrient limitation
CIP	Clean in place
cfu	Colony forming units
DNA	Deoxyribonucleic acid
eDNA	extracellular DNA
ELISA	Enzyme-linked immune-sorbent assay
EON	Biofilm formation on the stainless steel surface of an inadequately cleaned tanker that was left to stand empty overnight
EPS	Exopolysaccharides
GC	Gas chromatography
HPLC	High-performance liquid chromatography
MPCA	Milk plate count agar
MSI	Ministry of Science and Innovation
NB	Nutrient broth
NSLAB	Non-starter lactic acid bacteria
PAS	Planktonic growth in the absence of stainless steel surfaces
PCR	Polymerase chain reaction
pnpp	*p*-nitrophenol palmitate
PPS	Planktonic growth in the presence of stainless steel surfaces
PQQ-ADH	Pyrroquinoline quinine-dependent alcohol dehydrogenase
rDNA	Ribosomal DNA
RI	Refractive Index
RPM	Revolutions per minute
RSM	Reconstituted skim milk
SEB	Staphylococcal Enterotoxin B
SEM	Scanning electron microscopy
SMC	Biofilm formation on the stainless steel surface of an inadequately cleaned tanker with subsequent milk collection

SS	Stainless steel
TMC	Biofilm formation on the stainless steel surface after a typical 9.25 h of milk collection
Tris-HCl	Tris-hydrochloride
UV	Ultraviolet

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