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**The growth of thermophilic bacteria
in a milk powder plant
and the formation of spores in
biofilms of the dairy thermophile
*Anoxybacillus flavithermus***

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requirements for the degree of
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Abstract

Dairy production makes up 20% of New Zealand's export earnings, with whole milk powder being the number one dairy export. However, contamination of milk powder by spores of thermophilic bacteria is an ongoing problem in the manufacture of milk powder. These spores survive manufacturing processes, contaminate final product and have potential to spoil foods manufactured with milk powder. The main thermophilic organisms that cause concern in the New Zealand dairy industry are *Geobacillus* spp. and *Anoxybacillus flavithermus*. The vegetative forms of these organisms are able to grow within biofilms, but there is very little information as to the origin of their spores found in the product and the conditions under which they are produced. We have now monitored the dynamics and location of spore formation in the industrial and controlled laboratory settings.

A survey was undertaken at the Pahiatua milk powder manufacturing plant to determine the origin and rate of spore formation. The predominant sites of spore formation were the plate heat exchanger and evaporator. Spores began to develop approximately 11 h into an 18 h manufacturing run. The spores were identified as *Anoxybacillus flavithermus* and *Geobacillus* species.

To examine the dependence of spore formation on the development of the *A. flavithermus* biofilm under controlled laboratory conditions, a continuous flow reactor was used. The release of spores and vegetative cells into the milk was measured using change in impedance. Impedance change confirmed the presence of both vegetative cells and spores on stainless steel sample tubes.

At the end of an 8.5 h run at 55°C, using the continuous flow reactor, the total number of thermophilic bacteria released into the milk reached up to 10^6 cells mL⁻¹. At least 10 % of cells attached to the stainless steel surface were spores. These results indicate that spores form readily in biofilms of *A. flavithermus* believed to colonise the surface of the manufacturing plant. When the temperature of the continuous flow reactor was decreased to 48°C no spores were detected within the biofilm.

The results from this study have provided key information about where thermophilic spores form in a milk powder manufacturing plant and how biofilms of one of the typical thermophilic bacteria, *Anoxybacillus flavithermus*, develop. This knowledge will help the dairy industry to design strategies to prevent spore formation.

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Abbreviations

Aw	water activity
CFU	colony forming unit
CIP	clean-in-place
DSI	direct steam injection
EOR	end-of-run
HILLS	hygienic live line sampling
n/d	not determined
PHE	plate heat exchanger
RAPD	randomly amplified polymorphic DNA
SEM	scanning electron microscopy
SFB	static fluid bed
SSHE	scraped surface heat exchanger
TEM	transmission electron microscopy
T/S	total solids
UHT	ultra high temperature
UPGMA	unweighted pair group method using arithmetic averages
VF	vibrofluidiser
w/v	weight per volume

Definitions

Clean-in-place (CIP)	Cleaning regime after a manufacturing run
Direct steam injection (DSI)	A direct method of heat treatment where steam is injected into the milk.
Effect	A section of an evaporator that has the same boiling temperature
Fouling	Build up of milk deposits on the internal surface of dairy plants
Raw milk treatment	Separation, pasteurisation, and standardisation of raw milk
Orifice pans	Located at the top of the evaporator to distribute milk into the pass tubes
Pass	A section of the effect, in the evaporator, that is made up of a set of tubes that the milk passes through
Plate heat exchanger (PHE)	An indirect method of heat treatment that consists of a series of plates where the heating or cooling medium passes on one side, and the milk on the other.
Randomly amplified polymorphic DNA (RAPD) profiles	An electrophoretic gel pattern produced by amplifying random sections of the genome of interest with a short arbitrary primer of approximately 10 nucleotides.
Reactor	A laboratory system used to study biofilm development
Specification	Customers requirement of a product

Static fluid bed	A non-vibrating fluid bed located at the base of the main drier chamber, used for secondary drying of milk powder. Air is directed into the layer of powder at a shallow angle.
Total thermophile count	A plate count of bacteria that grow aerobically in milk and milk products at temperatures of 55 °C or higher (Fonterra, 2000b). This includes both obligate and facultative thermophiles.
Vibrofluidiser (or vibrating fluid bed)	A low frequency vibrating fluid bed that is used for either secondary or tertiary drying of milk powder. Air is directed into the layer of powder at a perpendicular angle.