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

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Jon Stuart Palmer

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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

Palmer J.S., Flint, S.H., Brooks, J. (2007) Bacterial cell attachment, the beginning of a biofilm. *Journal of Industrial Microbiology and Biotechnology*. **34**:577-588.

LIST OF PRESENTATIONS

Palmer J.S., Flint, S.H., Brooks, J., Schmid, J. (2005) Surface proteins of an adhesive thermophilic sporeforming bacilli from milk powder. *New Zealand Microbiological Society Conference,* Dunedin, New Zealand, 2005.

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g	acceleration due to gravity
AFM	atomic force microscope
BATH	bacterial adherence to hydrocarbons
bp	base pair
BLAST	basic local alignment search tool
BCA	bicinchoninic acid
BAP	biofilm associated protein
BSA	bovine serum albumin
°C	degrees Celsius
CHAPS	3-cholamidopropyl-dimethylammonio-1-propanesulfonate
CIP	cleaning in place
CID	collision induced spectra
CFU	colony forming units
Da	daltons
dNTP	deoxynucleotide triphosphate
DNA	deoxyribosenucleic acid
DLVO	Derjaguin, Landau, Verwey, Overbeek
DW	distilled water
DTT	Dithiothreitol
ESI	electrospray ionization
ESI-QUAD-TOF	electrospray ionization-quadrupole-time-of-flight
ESIC	electrostatic interactive chromatography
EB1	equilibration buffer I
EB2	equilibration buffer 2
EDTA	ethylenediamine tetraacetic acid
eDNA	extracellular deoxyribosenucleic acid
EPS	extracellular polysaccharide
g	gram(s)
g/l	grams per litre
HPLC	high performance liquid chromatography
h	hour(s)
HCl	hydrochloric acid
HIC	hydrophobic interactive chromatography
IPS	immobilised pH gradient
IDT	impedence detection time
IEF	isolectric electric focusing
pI	isolectric point
kb	kilo base pairs
kDa	kilodalton
LPS	lipopolysaccharide
L	litre(s)
LB	Luria-Bertani
$MgCl_2$	Magnesium chloride

$MgSO_4$	Magnesium sulphate
m/z	mass charge ratio
MALDI	matrix-assisted laser desorption/ionization
MATH	microbial adherance to hydrocarbons
µg	microgram(s)
µl	microlitre(s)
µm	micrometre(s)
µM	micromolar
mA	milliampere
mg	milligram(s)
ml	millilitre(s)
mm	millimitre(s)
mM	millimolar
min	minutes
M	molar
KH_2P0_4	monobasic potassium phosphate
ng	nanogram
nm	nanometer
ONPG	o-nitrophenyl-β-D-galactosidase
TEMED	N-N-N-N-tetramethylene diamine
ORF	open reading frame
OD	optical density
%	percentage
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
KCl	potassium chloride
RAPD	random amplified polymorphic DNA
RI	refractive index
rpm	revolutions per minute
NaCl	sodium chloride
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SD(s)	standard deviation(s)
SSP1	Staphylococcus surface protein 1
SSP2	Staphylococcus surface protein 2
MS/MS	tandem mass spectroscopy
TOF	time-of-flight
TEM	transmission electron microscopy
TAE	tris-acetate-EDTA
Tris	tris(hydroxymethyl)aminomethane
TE	tris-HCl-EDTA
TSA	tryptic soya agar
TSB	tryptic soya broth
2D-PAGE	two dimensional-polyacrylamide gel electrophoresis
UV	ultraviolet
v/v	volume/volume

V	volts
VH	volt hours
w/v	weight/volume
XPS	x-ray photoelectric spectroscopy

Amino acids

A	alanine
E	glutamic acid
H	histidine
L	leucine
P	proline
S	serine
V	valine
C	cysteine
F	phenylalanine
I	isoleucine
M	methionine
Q	glutamine
T	threonine
Y	tyrosine
D	aspartic acid
G	glycine
K	lysine
N	asparagine
W	tryptophan

Nucleotides

A	adenosine
C	cytidine
G	guanosine
T	thymidine