Germination of psychrotolerant clostridia responsible for red meat spoilage

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Abstract

Psychrotolerant clostridia are responsible for spoilage of fresh chilled vacuum-packed red meat (beef, lamb and venison). Red meat is one of New Zealand's primary exports, and spoilage results in financial loss. Spoilage by psychrotolerant clostridia is difficult to control due to the ability of these bacteria to grow at cold temperatures, down to -1.5 °C. They can also form spores that have increased resistance to heat, chemicals, oxygen and desiccation compared to vegetative bacterial cells. As clostridia are strict anaerobes, it is considered highly likely that initial contamination of meat is primarily with spores. The main objective of this work was to determine the triggers of germination of spores, of those psychrophilic and psychrotrophic clostridia, associated with spoilage of New Zealand red meat.

Germination of psychrotolerant clostridia was studied using a range of techniques including molecular, *in vitro,* and on meat methods. In this study *in vitro* germinant systems were identified for *Clostridium frigidicarnis,* and a New Zealand species designated LA1, consisting of lactate in combination with an amino acid. Some of the amino acids identified, including valine and cysteine, are naturally present on the surface of red meat. Failure to chill to, or maintain meat at, the recommended temperature, of -1.5 ˚C and a pH of above 5.5 were identified as being important factors leading to spoilage by *Cl. frigidicarnis*. Germination in *Clostridium estertheticum* was extremely poor in media, compared with meat slurry or fresh meat, preventing the identification of a specific germinant system(s), and indicating a non-nutrient factor may be involved. Two distinct nonchemical interventions, hot water wash (HWW) and cold water wash (CWW), were found to reduce spoilage of vacuum-packed chilled lamb inoculated with spores of *Cl. estertheticum*. Vegetative cells of psychrotolerant clostridia survived exposure to air longer than expected, upwards of seven days in the case of *Cl. estertheticum* subsp. *estertheticum,* suggesting that they play a greater role in initial contamination of meat than originally thought. From an industry point of view the results highlight the importance of preventing initial contamination and proper chilling, as well as the need for further investigation of HWW and CWW interventions.

List of Publications

Adam, K.H., Flint, S.H. and Brightwell, G. (2010). Psychrophilic and psychrotrophic clostridia: sporulation and germination processes and their role in the spoilage of chilled, vacuum-packaged beef, lamb and venison. *International Journal of Food Science & Technology.* **45**:1539-1544.

Adam, K.H., Brunt, J., Brightwell, G., Flint, S.H. and Peck, M.W. (2011). Spore germination of the psychrotolerant, red meat spoiler, *Clostridium frigidicarnis*. *Letters in Applied Microbiology*. **53**:92-97

Adam, K.H., Flint, S.H. and Brightwell, G. (2012). Mechanisms and reduction of spoilage of chilled vacuum-packed lamb by psychrotolerant clostridia. *Meat Science*. In Press.

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List of Abbreviations

APC	aerobic plate count
AFR*	L-alanine, L-phenylalanine, L-arginine and $NaHCO_3$
AGFK	L-asparagine, D-fructose, D-glucose and potassium ions
bCMM	biphasic cooked meat medium (recipe section 3.4)
BHI	brain heart infusion
BLAST	basic logical alignment search tool
bp	base pair
°C	degrees Celsius
CBA	Columbia Blood Agar
CFU	colony forming units
CLEs	cortex lytic enzymes
dCMM	dried cooked meat medium
fCMM	fresh cooked meat medium (recipe section 3.4)
CWW	cold water wash
DIG-	dioxigenin-
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DPA	pyridine-2, 6-dicarboxylic acid (dipicolininc acid)
F	variance of the means
g	acceleration due to gravity
g	gram(s)
GR	germinant receptor
GMP	good management practice
h	hour
mH_2O	Milli-Q water
uH_2O	Gibco™ UltraPure™ distilled, DNAse and RNAse free water
HCl	hydrochloric acid
HWW	hot water wash
ITS	internal transcribed spacer
KH_2PO_4	monobasic potassium phosphate

kmh^{-1}	kilometre per hour
L	litre
log	logarithm
$MgCl_2$	magnesium chloride
mg	milligram
ml	millilitre
mM	millimolar
min	minute
mol	molar
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
ng	nanogram
O_2	oxygen
OD	optical density
PCR	polymerase chain reaction
POAA	peroxyacetic acid
PYGS	peptone, yeast extract, glucose, starch medium (recipe section 3.4)
rpm	revolutions per minute
s	second
SASP	small, acid-soluble, spore proteins
SDS	sodium dodecyl sulphate
SSC	saline-sodium citrate
sp.	species
subsp.	sub species
Tris	tris(hydroxymethyl)aminomethane
µg	microgram
µl	microliter
µmol	micromolar
µm	micrometre
UV	ultraviolet
%	percentage

Nucleotides

A	adenine
C	cytidine
G	guanosine
T	thymadine

Bases in degenerate primers

R	A + G
Y	C + T
M	A + C
K	G + T
S	G + C
W	A + T
H	A + T + C
B	G + T + C
D	G + A + T
V	G + A + C
N	A + C + G +T