A Novel Approach for Controlling Foodborne Pathogens Using Modified Atmosphere and *Lactobacillus reuteri* DPC16

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Abstract

The current trend of increasing demand for minimally processed food requires more effective preservation technologies than are presently used. In this study, an investigation has been made into a novel strategy to control some common foodborne pathogens, and therefore, to provide an alternative means for enhancing the safety and extending the shelf lives of food products.

Modified atmosphere is able to extend the shelf life of seafood and meat products. In this study, a simulated controlled atmosphere (CA) broth system was used to investigate the potential of a modified atmosphere rich in CO_2 at a concentration of 40%, supplemented with N_2, to control common foodborne pathogens, such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, *Staphylococcus aureus* and *Vibrio parahaemolyticus*. Controlled atmosphere significantly reduced the exponential growth rates of all tested pathogens, while the effects on other growth parameters (eg. lag phase duration and maximum population density) depended on the individual species and the specific growth conditions. The CA significantly extended the lag phase durations of *S. aureus* and *V. parahaemolyticus* at 20^{o}C at both pH 6.3 and 6.8, and that of *L. monocytogenes* at both 7^{o}C and 20^{o}C, and at both pH 6.3 and 6.8. The CA also significantly lowered the maximum population densities of *S. aureus* and *V. parahaemolyticus* at 20^{o}C, at pH 6.3 and 6.8, *S.* Typhimurium at pH 6.8, and *L. monocytogenes* at pH 6.3 and 7^{o}C. *E. coli* O157:H7 and *S.* Typhimurium were more resistant to the inhibitory effect of the CA, while *S. aureus* and *V. parahaemolyticus* were most sensitive. The inhibitory effect of CA was due mainly to the extensions of the lag phase duration and the reduction of the exponential growth rates of the test pathogens. This study confirms other studies that CA as a means for food preservation provides potential to control foodborne pathogens and therefore enhance the safety of a food product.

The use of lactic acid bacteria (LAB) in controlling spoilage microorganisms and pathogens in foods has been a popular research theme worldwide. In this study, the antimicrobial effects of 18 lactic acid bacteria strains were evaluated *in vitro*, with emphasis on the most effective strain, the newly characterised *Lactobacillus reuteri* DPC16. The results demonstrated antagonistic effects of many strains against *L. monocytogenes*, *E. coli* O157:H7, *S.* Typhimurium and *S. aureus*. *L. reuteri* DPC16 showed the strongest antimicrobial activity against the tested pathogens including both Gram-positive and Gram-negative bacteria. Co-cultivation of *L. reuteri* DPC16, and co-incubation of its spent culture supernatant (DPC16-SCS), with the pathogens have demonstrated that the antimicrobial effect is bactericidal and valid at pH 4 - 6.5 and at a temperature as low as 10^{o}C. Further characterisation of the antimicrobial effect of *L. reuteri* DPC16 showed it to be mainly due to the presence of reuterin (β-hydroxypropionaldehyde), although lactic acid may have also played a role. These characteristics of *L. reuteri* DPC16 and its metabolite reuterin make it an unique and potent candidate as a biopreservative to control both Gram-positive and Gram-negative bacteria in foods.

The combination of *L. reuteri* DPC16 and CA was assessed for its inhibitory effect on *L. monocytogenes* using DPC16-SCS and the fermentative supernatant of *L. reuteri* DPC16 from a glycerol-water solution (DPC16-GFS). The results showed that both of these supernatants, at 25 AU/mL, in combination with CA (60% CO_2:40% N_2) had a combined inhibitory effect on *L. monocytogenes* which could not be achieved by any one of the individual factors alone.

Analysis of the levels of expression of some stress response genes of *L. monocytogenes*, after growth in the presence of *L. reuteri* DPC16 supernatant and/or CA, showed that the expression of some genes was affected including genes *betL*, *gbuA* and *opuCA* responsible for osmosis adaptation and genes *gadA*, *gadB* and *gadC* responsible for acid tolerance. Induction of *gbuA*, *gadB* and *gadC* by the culture supernatant suggests activation of osmotic and acid adaptation and that these genes play a major role in the culture supernatant-induced stresses.

An investigation was also carried out to determine if the changes in gene expression conferred a cross-protection to heat. The result showed that the survival of *L. monocytogenes* grown in the presence of the culture supernatant and CA was significantly increased after exposure to heat treatment at 56^{o}C, suggesting that a cross-protection to thermal stress had been induced.

Based on these findings it is proposed that a comprehensive novel strategy incorporating both *L. reuteri* DPC16 or its fermentative products and a modified atmosphere rich in CO_2 could be developed to potentially control foodborne pathogens in food products.

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Abbreviations

A	Adenine
AH	Acid habituation
ANOVA	Analysis of variance
AR	Acid resistance
ASPs	Acid shock proteins
AT	Acid tolerance
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
ATR	Acid tolerance response
bBHI	Buffered BHI
bMMRS	Buffered MMRS
bTSBS	Buffered TSBS
BLAST	The Basic Local Alignment Search Tool
C	Cytosine
C	*Clostridium*
CA	Controlled atmosphere
CDC	Centres for Disease Control and Prevention of USA
CFU/mL	Colony forming units per milliliter
CO_2	Carbon dioxide
CO	Carbon monoxide
cDNA	Complementary deoxyribonucleic acid
Bifido	*Bifidobacterium*
BLIS	Bacteriocin-like inhibitory substance
BHI	Brain Heart Infusion
bp	Base pair
^{o}C	Degree Celsius
cm	centimetre
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotides: dATP, dCTP, dGTP and dTTP

DPC16-GFS	*L. reuteri* DPC16 glycerol fermentative solution
DPC16-SCS	*L. reuteri* DPC16 spent cultural supernatant
DTT	Dithiothreitol
E	*Escherichia*
EDTA	Ethylenediamine tetra-acetic acid
e.g.	*exempli gratia*, mean "for example"
EGR	Exponential growth rate
Entero	*Enterococcus*
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration of the US
FSANZ	Food Standard Australia New Zealand
g	Gram or acceleration due to gravity (9.8 m/s^2)
G	Guanine
GABA	γ-aminobutyrate
GAD	Glutamate decarboxylase
GI	Gastrointestinal
GRAS	generally recognised as safe
GroEL	chaperonin, a heat shock protein
GT	Generation time
IU	International unit
h	Hour
HACCP	Hazard analysis and critical control points system
H_2O	Water
HAV	Hepatitis A virus
HCl	Hydrogen chloride
H_2O_2	Hydrogen peroxide
HSPs	Heat shock proteins
KCl	potassium chloride
Kg	kilogram
kGy	kiloGrays
L	*Listeria* or *Lactobacillus*
L	Litre

Lb	*Lactobacillus*
Leuco	*Leuconostoc*
L-lactic acid	Levorotatory isomer of lactic acid
LAB	Lactic acid bacteria
Log	Logarithm
LPD	Lag phase duration
M	Molar
MA	Modified atmosphere
MAP	Modified atmosphere packaging
MDOs	Membrane-derived oligosaccharides
mg	Milligram
MPa	Million pascal
MPD	Maximum population density
MRS	de Man, Rogosa, Sharpe
MMRS	Modified MRS
MRSg	MRS broth supplemented with 250 mM glycerol
µg	Microgram
µL	Microlitre
µm	Micrometre
µM	Micromolar
mm	Millimetre
mM	Millimolar
min	Minute
mL	Millilitre
MviA	a protein of 38 kDA, the product of the mouse virulence regulatory gene *MviA*
N_2	Nitrogen
Na	Sodium
NAG	non-agglutinable *vibrio*
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information

NCV	non-cholera *vibrio*
NFPA	The National Food Processors Association, USA
NLV	Norwalk-like virus
NoV	Norovirus
OD	Optical density
O_2	Oxygen
OpuC	Carnitine transporter
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
P	P value
pH	$-Log[H^+]$
ppm	Part per million
%	Per cent
% v/v	per cent by volume/volume
% w/v	per cent by weight/volume
rmp	Rotation per minute
R	Purines
RNA	Ribonucleic acid
RT	Reverse transcription
rDNA	rRNA gene
rRNA	Ribosomal RNA
S	*Salmonella* or *Staphylococcus*
σ^B	Sigma B factor
σ^H	Sigma H factor
σ^S	RpoS, the alternative sigma factor ς^{38}
SCS	Spent culture supernatant
sec	Second
SO_2	Sulphur dioxide
spp	Species
SRSVs	Small round structured viruses
SSE	lowest sum of squares error
T	thymine or temperature

TAE	Tris-Acetate-EDTA
Tris	Tris (hydroxymethy)amiomethane
TSA	Trypticase soy agar
TSB	Trypticase Soy Broth
TSBS	TSB supplemented with 3% sodium chloride
U	unit
UV	Ultra violet light
V	*Vibrio*
vs	Versus
WHO	The World Health Organisation of the United Nations

Amino Acid Abbreviations

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Iosleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V