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An immobilised cell system for the  
delivery of functional *Lactobacillus*  
*reuteri* DPC16 cells to their target site  
in a simulated gastrointestinal tract

Qian Zhao

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2012

An immobilised cell system for the  
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Qian Zhao

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## List of publications

1. Functional properties of free and encapsulated *Lactobacillus reuteri* DPC16 during and after passage through a simulated gastrointestinal tract (published by *World Journal of Microbiology and Biotechnology*, 2012, 28(1), 61-70.);
2. Viability and delivery of immobilised *Lactobacillus reuteri* DPC16 within calcium alginate gel systems during sequential passage through simulated gastrointestinal fluids (published by *Beneficial Microbes*, 2011, 2(2), 129-138.);
3. The effect of cell immobilisation on the antibacterial activity of *Lactobacillus reuteri* DPC16 cells during passage through a simulated gastrointestinal tract system (submitted to *World Journal of Microbiology and Biotechnology* on 18<sup>th</sup> April, 2012).

## Abstract

The objective of this study was to design and produce calcium alginate beads that can deliver immobilised cells of *Lactobacillus reuteri* DPC16 to a target site of the colon in the gastrointestinal (GI) tract, without any diminution of their important physiological characteristics. Several factors that might affect the effectiveness of calcium alginate beads for the cell delivery were investigated, using an *in vitro* GI tract model to simulate the conditions within the tract. Firstly, by varying the concentration of alginate at a constant concentration of  $\text{CaCl}_2$ , and combining the system with gelatin, chitosan or skim milk, the survival of immobilised DPC16 cells in simulated gastric fluid (SGF) was observed. Secondly, the physical stability of calcium alginate beads containing skim milk was observed during sequential incubation in the GI fluids using optimal concentrations of alginate. Finally, the survival of DPC16 cells immobilised within alginate beads containing skim milk was monitored when the beads were incubated for different times during sequential exposure to the simulated fluids. The results demonstrated that non-encapsulated DPC16 cells were sensitive to an acidic environment, and no viable cells were detected after 90 min exposure in SGF (pH 1.2). After appropriate experimentation, an alginate concentration of 3% (w/v) was deemed to be the optimum value and was used in subsequent investigations. When skim milk (8% (w/v)) was added to the alginate solution, the cell survival in SGF was improved markedly. The optimal concentration of calcium chloride was 0.3 M, based on the beads maintaining their integrity in SGF and simulated intestinal fluid (SIF) while disintegrating in simulated colonic fluid (SCF) to release viable cells. Hence, the beads made from 3% alginate, 8% skim milk and 0.3 M  $\text{CaCl}_2$  proved to be an effective delivery and release system for DPC16 cells.

*L. reuteri* DPC16 has strong antimicrobial activities against pathogens, due mainly to its ability to produce reuterin. Hence this and other functional properties of the bacterial cells were studied before and after passage through the GI tract. The cells that were recovered after release from the alginate beads in the SCF showed no diminution in functional properties, including their growth kinetics, ability to adhere to epithelial cells and ability to inhibit the adhesion of *E. coli* to epithelial cells. However, the bacteriostatic and bactericidal properties of the recovered cells against some pathogens

were significantly greater ( $P < 0.05$ ) than those of the original cells. Production of reuterin by the recovered cells was significantly greater ( $P < 0.05$ ) than that of the original cells when cultured in MRS medium in the absence of its metabolic precursor, glycerol. The results demonstrate significant ( $P < 0.05$ ) consequences for the application of the encapsulation technique to protect and/or enhance the functional properties of the probiotic cells.

Subsequently, an investigation was carried out to find the reason for the antimicrobial activity enhancement. By recovering cells from different stages of the immobilisation and delivery process and examining them for their antimicrobial properties, it was found that it was the immobilisation process *per se*, rather than passage through the simulated gastrointestinal fluids, that caused the enhancement of antimicrobial activity, and that this was related to increased activity of the enzyme (diol dehydratase) that is responsible for reuterin production from glycerol.

Finally, it was demonstrated that freeze-drying of the alginate beads was not an appropriate storage technique as it resulted in a significant ( $P < 0.05$ ) diminution of the antimicrobial activities.

Based on these findings it is confirmed that the alginate-skim milk- $\text{CaCl}_2$  immobilisation system is an effective and efficient method, not only for protecting the viability of DPC16 cells, but also for maintaining the physiological characteristics.

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## Abbreviations

3-HPA	3- hydroxypropionaldehyde
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BSA	Bovine serum albumin
BHI	Brian heart infusion broth
cFDA	carboxy fluorescein di-acetate
c.f.u.	Colony forming units
DMEM	Dulbecco's modified Eagle's medium
EDTA	Ethylenediamine tetra- acetic acid
FAO	Food and Agriculture Organisation of the United Nations
g	gram
GI tract	Gastrointestinal tract
h	hour
Hb	haemoglobin
IDF	International Dairy Federation
LAB	Lactic acid bacteria
MEM	Modified Eagle's medium
min	minute
MRS	De Man, Rogosa, Sharpe
MRSg	MRS broth supplemented with 250 mM glycerol
OD	Optical density
P	Provability value
PBS	Phosphate buffered saline
PI	propidium iodide
rpm	Revolutions per minute
SCFAs	Short chain fatty acids
SCF	Simulated colonic fluid
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	second

## Abbreviations

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SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
TCA	Trichloroacetic acid
Tris	Tris (hydroxymethyl) amiomethane
UV	Ultra violet light
WHO	The World Health Organisation of the United Nations