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Publication date:
2010

Document version
Peer reviewed version

DEPARTMENT OF FOOD SCIENCE
FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

Expression of *luxS* gene involved in quorum sensing in *Lactobacillus acidophilus* NCFM after passage through an *in vitro* digestion model

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Introduction

Within recent years, there has been an increasing interest in discovering the beneficial effects of probiotics and the mechanisms by which probiotics interact with the host and the gut microbiota. One of the mechanisms that bacteria interact or communicate with each other is quorum sensing. Quorum sensing is cell-to-cell signalling through the production, secretion and detection of small signal molecules called autoinducers. The aim of the present study was to investigate the transcription of the *luxS* gene involved in quorum sensing in probiotic strain *Lactobacillus acidophilus* NCFM after passage through an *in vitro* digestion model, which could have effect on cell-to-cell communication between microorganisms of gut microbiota.

Materials and Methods

Strain and Growth condition
Overnight culture of *L. acidophilus* NCFM was inoculated (1.0%) in 50 ml MRS broth for 16 h at 37°C with the final pH of 4.2 and centrifuged and used for the experiments, or inoculated (10.0%) in 11.0% skim milk for 28 h at 37°C with final pH of 4.2 and 5 g of the culture was utilized.

***In vitro* Digestion Model**
An *in vitro* digestion model of the upper gastrointestinal tract developed by Oomen et al. (2003) was used (Fig.1), including three compartments; mouth: saliva juice (6 ml, pH 6.5 for 5 min), stomach: gastric juice (12 ml, final pH 3.5 for 2 h) and small intestine: duodenal and bile juices (12 and 6 ml, respectively, final pH 6.5 for 2 h), incubation at 37°C with rotation head-over-heels. Samples were taken at t=0 and 30 min intervals for determination of survival and gene expression analysis using Taqman-based quantitative real-time PCR. The housekeeping gene 23S rRNA was used for normalisation.

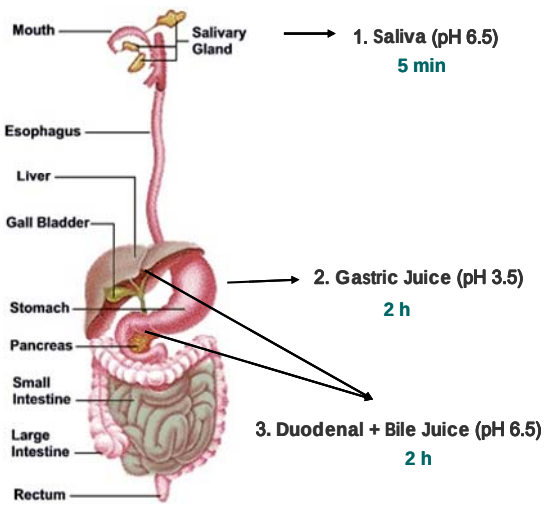

Fig. 1 Schematic diagram of the *in vitro* digestion model

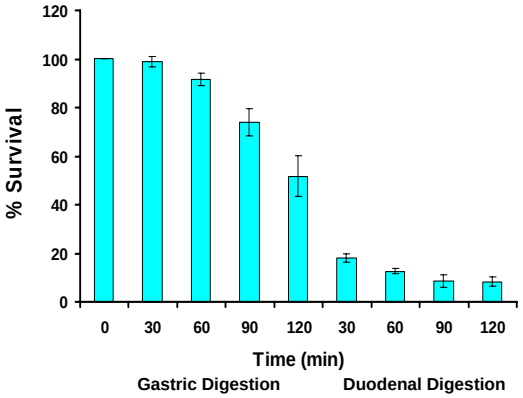

Fig. 2 Survival of *L. acidophilus* NCFM cells grown in MRS after passage through the *in vitro* digestion model

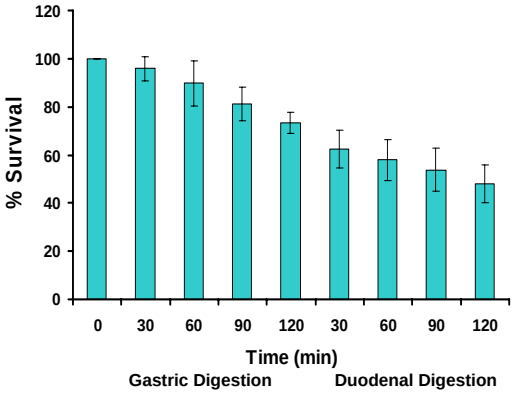

Fig. 3 Survival of *L. acidophilus* NCFM cells grown in skim milk after passage through the *in vitro* digestion model

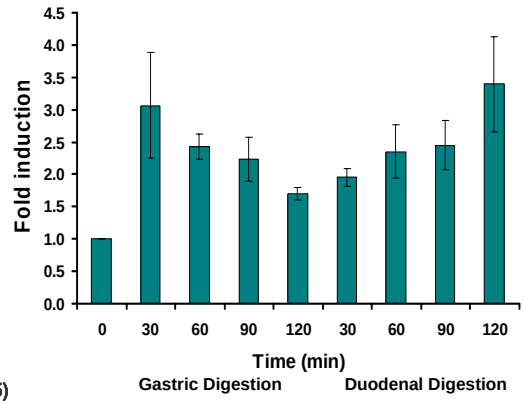

Fig. 4 Expression of the *luxS* gene in *L. acidophilus* NCFM cells grown in skim milk after passage through the *in vitro* digestion model

Results

1. Survival of cells grown in MRS broth reduced significantly after 1 h incubation in the gastric juice (pH 3.5) and only 52% of cells survived after 2 h. Following 30 min incubation of cells in duodenal and bile juices a huge decrease in survival was observed (18%) and it reduced to 8% after 2 h (Fig. 2).

2. Survival of cells grown in skim milk was significantly higher after 2 h incubation in gastric juice (73%) and during incubation in the duodenal and bile juices (48% after 2 h) compared to the cells grown in MRS broth (Fig. 3).

3. In cells grown in skim milk, the transcription of the *luxS* gene increased 3.0 fold after 30 min incubation in the gastric juice and reduced afterwards. The transcription increased again after incubation of cells in duodenal and bile juices. The highest transcription (3.4 fold) was observed after 2 h incubation in duodenal and bile juices (Fig. 4). Cells grown in MRS without protection of the food matrix were under severe stress during incubation at the harsh conditions of the digestion model, and therefore gene expression was highly unstable and could not be determined.

Conclusions

The *luxS* gene of *L. acidophilus* NCFM was up-regulated at the stress conditions of the *in vitro* digestion model which shows that passage through the gastrointestinal tract has significant role in the induction of *luxS* gene in this probiotic, which has been shown to be involved in regulation of different behavior in probiotic lactobacilli. Thus this up-regulation might have significant effect in probiotic functionality and also cell-cell communication between microorganisms of gut ecosystem. In addition it was shown that the food matrix has an important role in the survival of the cells after stress conditions of the gastrointestinal tract.

Acknowledgements

This study is financed as a PhD grant by the University of Copenhagen, Faculty of Life Sciences.

Reference

Oomen AG, Rompelberg CJ, Bruil MA, Dobbe CJ, Pereboom DP, Sips AJ (2003). Development of an *in vitro* digestion model for estimating the bioaccessibility of soil contaminants. Archives of environmental contamination and toxicology 44:281-7.