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Quantitative expression analysis of the *luxS* gene after exposure to low pH in *Lactobacillus acidophilus* NCFM and *Lactobacillus rhamnosus* GG

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Introduction

The ability to respond to various environmental stresses such as low pH is an important factor in lactobacilli for their function in food fermentation and as probiotics. LuxS-mediated quorum sensing mechanism, which is based on the production of universal signalling molecule called autoinducer-2 (AI-2) by the activity of the LuxS enzyme, regulates a variety of adaptive processes in different bacteria. The aim of this study was to investigate whether the expression of the *luxS* gene was influenced by low pH stress in two well-documented lactobacilli, *Lactobacillus acidophilus* NCFM and *Lactobacillus rhamnosus* GG. Furthermore, the effect of acid adaptation on expression of the *luxS* gene and AI-2 activity was studied.

Materials and Methods

Strains:
L. acidophilus NCFM and *L. rhamnosus* GG

Growth condition:
Modified MRS medium (glucose was replaced by galactose), 37°C and anaerobic

Investigation of expression of the *luxS* gene:
Expression of the *luxS* gene was determined after exposure of mid-exponential acid adapted (1 h at pH 5.0) and non-adapted (1 h at pH 6.5) cells to pH 4.0 and pH 6.5 (as control) using (Taqman based) quantitative Real-Time PCR.

Investigation of AI-2 activity:
Production of AI-2 by acid adapted and non-adapted cells after exposure to pH 4.0 and pH 6.5 (as control), was determined using the *Vibrio harveyi* bioluminescence assay, which relies on expression of the luminescence genes in *V. harveyi* reporter strain (BB170) in response to AI-2.

Results

1. No obvious up-regulation of the *luxS* gene was observed after exposure to pH 6.5 in both strains.

2. Exposure to pH 4.0 resulted in significant up-regulation of the *luxS* gene in both strains. The up-regulation was higher in *L. acidophilus* NCFM compared to *L. rhamnosus* GG.

3. The up-regulation of the *luxS* gene was reduced by acid adaptation in both strains.

4. AI-2 production was significantly higher after exposure to pH 4.0 compared to pH 6.5 in both strains and decreased by acid adaptation. These results validated the results from gene expression studies.

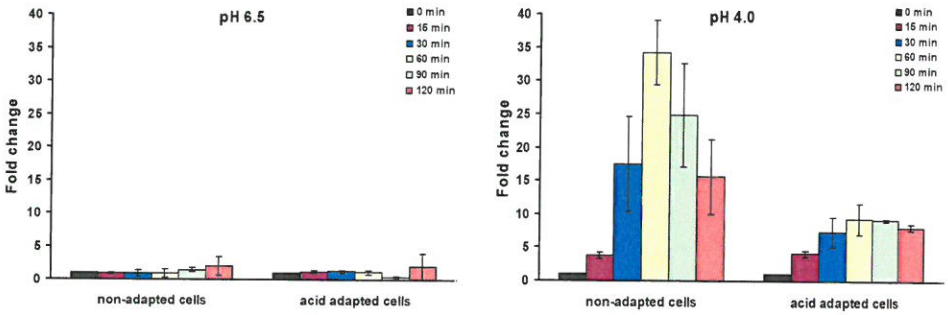

Fig. 1a Expression of the *luxS* gene after exposure of acid adapted and non-adapted cells of *L. acidophilus* NCFM to pH 4.0 and 6.5.

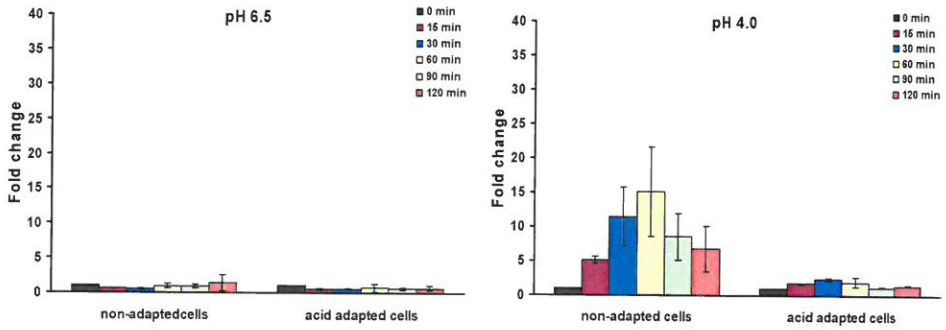

Fig. 1b Expression of the *luxS* gene after exposure of acid adapted and non-adapted cells of *L. rhamnosus* GG to pH 4.0 and 6.5.

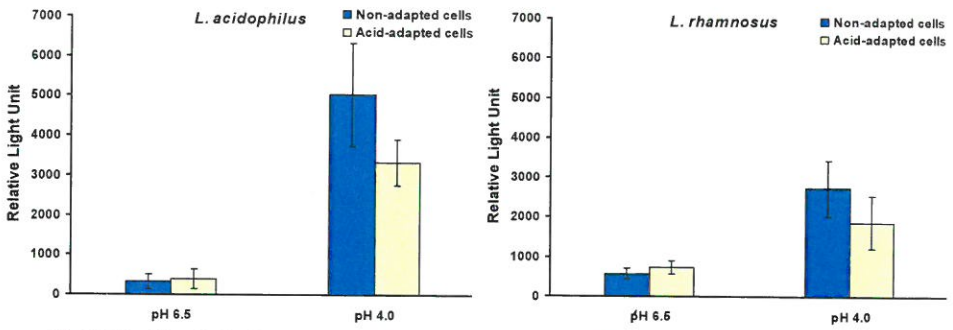

Fig. 2 AI-2 activity of acid adapted and non-adapted cells of *L. acidophilus* NCFM and *L. rhamnosus* GG after 1 h exposure to pH 4.0 and pH 6.5.

Conclusions

The *luxS* gene might be involved in regulation of low pH stress in *L. rhamnosus* GG and *L. acidophilus* NCFM. It is possible that passage through the stomach and the gastrointestinal tract may increase LuxS-mediated quorum sensing which could have an effect on the cell-to-cell communication in the gastrointestinal tract.

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