Influence of pH on gene expression in *Lactobacillus parabuchneri* FAM21731

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Introduction

Biogenic amines in cheeses can occur due to amino acid decarboxylase activity of bacteria. Histamine is the most important biogenic amine in cheese and can cause health problems after ingestion. It is produced by histidine decarboxylase (*hdcA*), positive lactic acid bacteria. In previous studies *hdcA* containing bacteria were isolated from various raw milk cheeses. All isolates belonged to the species *Lactobacillus parabuchneri*, which are heterofermentative lactic acid bacteria. Strategies to prevent accumulation of histamine in raw milk cheeses could be developed if the metabolism of these bacteria is known in more detail.

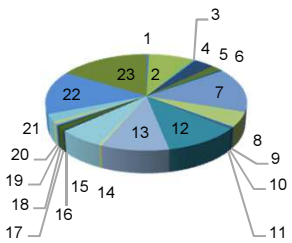
In previous cheese making experiments it was shown that *Lb. parabuchneri* forms 1,2-propanediol. In this study we investigated the linkage between histamine and 1,2-propanediol production.

Therefore we studied the influence of two different pH (3.8 and 6.8) on gene expression. *Lb. parabuchneri* was grown at 30°C in modified MRS-medium containing 45 mM D-, L-lactate and 2.7 g/L glucose. After three days of growth bacteria were harvested for RNA isolation. Isolated RNA was used for whole transcriptome analysis using next generation sequencing (Ion Torrent PGM).

Results

Transcriptome Analysis

Overall transcriptome analysis shows 419 significantly regulated genes (p-value<0.05, n=3) of which 228 were up- and 191 down-regulated at pH 3.8 (Fig. 1).



(1) anatomical structure development
(2) biosynthetic process
(3) carbon utilization
(4) catabolic process
(5) cellular component biogenesis
(6) cellular component organization
(7) cellular metabolic process
(8) establishment of localization
(9) macromolecule localization
(10) methylation
(11) multi-organism cellular process
(12) nitrogen compound metabolic process
(13) organic substance metabolic process
(14) primary metabolic process
(15) regulation of biological process
(16) regulation of biological quality
(17) response to chemical stimulus
(18) response to external stimulus
(19) response to stress
(20) single organism signaling
(21) single-organism cellular process
(22) single-organism developmental process
(23) single-organism metabolic process

Figure 1
Distribution of GO-terms (Gene Ontology) of the significant regulated genes gives an overview on the biological processes putatively affected by pH.

Read mapping for genes of interest

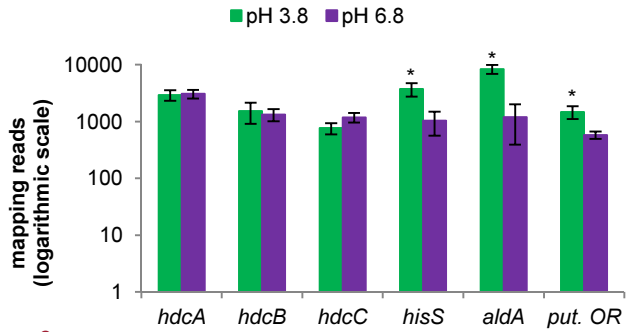


Figure 2
Of the genes belonging to the histidine decarboxylase gene cluster (*hdcA*, *hdcB*, *hdcC* and *hisS*) only *hisS* is significantly up-regulated indicating no influence of pH on histamine production.
Furthermore we found *aldA* (lactaldehyde dehydrogenase) and a putative oxidoreductase (*put. OR*) **up-regulated** ($p<0.05$) at pH 3.8. The gene products might play a role in the formation of 1,2-propanediol.

Formation of 1,2-propanediol

The formation of 1,2-propanediol was only detected in the culture supernatant of cells grown at pH 3.8 (Fig. 3, left). Interestingly the culture supernatant at pH 3.8 contained less L-lactate (Fig. 3, right) indicating that this compound is an intermediate in 1,2-propanediol formation.

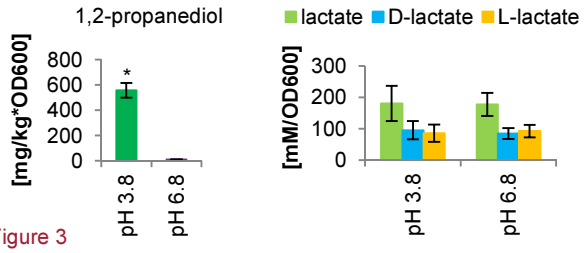
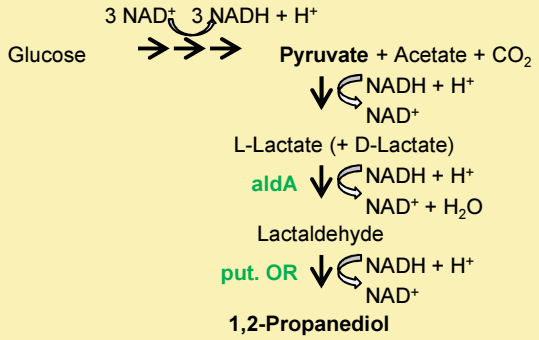


Figure 3
Level of 1,2-propanediol (left) and lactate (right) in the culture supernatants at pH 3.8 and pH 6.8 (n=6).

Conclusion and Outlook

Transcriptome analysis showed the influence of pH on the expression of 419 genes in *Lb. parabuchneri* (Fig. 1). Three genes of the histidine decarboxylase cluster are not concerned (*hdcA*, *hdcB* and *hdcC*), while *hisS* is up-regulated (Fig. 2). Furthermore there are two genes (*aldA* and *put. OR*) up-regulated which might play a role in 1,2-propanediol formation (Fig. 2). 1,2-Propanediol was only formed at pH 3.8 and there are hints that only L-lactate is used for its formation (Fig. 3).
Out of these data we propose the following pathway for 1,2-propanediol formation:



In further experiments we want to clone the genes probably linked to 1,2-propanediol formation. Additionally feeding experiments with ^{13}C-glucose and ^{13}C-lactate are planned to further investigate the hypothesis that L-lactate is the intermediate in 1,2-propanediol formation.

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