Isolation and characterization of a cysteine biosynthetic gene in *Lactobacillus casei* encoding cysteine lyase and synthase activity

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Abstract

Lactic acid bacteria are widely used as starter and nonstarter cultures in the dairy industry. In cheese they play an essential role in the degradation of casein-derived amino acids which are converted to essential flavour compounds mainly by enzymatic pathways. Metabolism of sulfur-containing amino acids in *Lactobacillus casei* has not been extensively studied. Recently, we observed that several *Lactobacillus casei* strains isolated from Gruyère cheese grew in a chemical defined medium containg methionine as the sole sulfur source (Irmler *et al.*, 2008). This indicated that cysteine biosynthesis must occur. Data about cysteine biosynthesis is limited in *Lactobacillus casei* and our first goal was the characterization of a gene potentially encoding cysteine synthase (*cysK*). The *cysK* gene was cloned from *Lactobacillus casei* FAM 18110 isolated from Swiss Gruyère cheese and a recombinant protein was overproduced in *E.coli*. It was shown that the recombinant enzyme on one hand synthesized cysteine from O-acetylserine and sodium sulfide and on the other hand produced hydrogen sulfide from L-cysteine in the presence of a nucleophile. Km and Vmax values of the enzyme for both activities were determined. The role of *cysK* gene product in role in cysteine biosynthesis was confirmed by complementation experiments with the cysteine auxotroph strain *Escherichia coli NK3*.

Background

L-Cysteine is essential for the synthesis of proteins, secondary metabolites, coenzymes and related compounds. To our knowledge, the free cysteine content in cheese has never been determined and cysteine is present in the caseins at a lower concentration than methionine. Therefore, we think that lactic acid bacteria growing in cheese are actively synthesizing cysteine.
Lactobacillus casei is a predominant species found in cheese after ripening. Genomic data of *L. casei* ATCC 334 show that the gene LSEI_0480 encodes a putative cysteine synthase (CysK, EC 2.5.1.47). This enzyme could synthesize cysteine from O-acetylserine and sulfide. However, a gene encoding the enzyme to synthesize O-acetylserine is missing in ATCC 334. It was reported that the cysteine synthase from *Bacillus subtilis* possesses cystathionine β-synthase (CBS; EC 4.2.1.22) activity (Hullo et al., 2007). Additionally, it was shown that cysteine synthases are also involved in the catabolism of cysteine (Fukamachi et al. 2002) which means that the gene could play a role in flavor formation in cheese. Since the catalytic activity and substrate specificity of CysK from *L. casei* is not predictable from the genetic information, the aim of the present study was to investigate the enzymatic properties of CysK and clarify its role in sulfur metabolism.

Results

Expression and purification of *L. casei* CysK

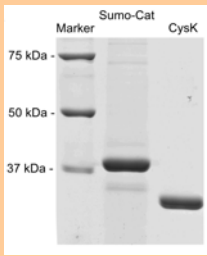

Figure 1 SDS PAGE analysis of purified recombinant CysK
CysK was fused to a His-tag and expressed in *E.coli* BL21. The His-tag allowed purification of the recombinant protein by Ni^{2+}-affinity chromatography. Denaturing SDS-PAGE analysis showed that the recombinant CysK had a size of 32.1 kDa (expected 34.8 kDa) and a high degree of purity. SUMO-CAT fusion protein was used as a control and showed a size of 37.7 kDa (expected 39.0 kDa).

Complementation experiment

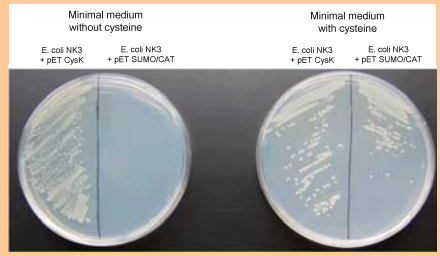

Figure 2 In vivo activity of CysK
Analysis of enzymatic acitivies showed that CysK catalyzes the synthesis of cysteine. The *in vivo* activity of CysK was proved by complementation of the cysteine auxotroph *E. coli* NK3. NK3 was transformed with plasmid carrying *cysK*. Transformants grew on minimal medium lacking cysteine (Petri dish on the left hand side) whereas NK3 transformed with control vector could not.

Enzymatic assays

Kinetic parameters / ***Substrates***	***Km (mM)***	***Vmax (mmol/(min*mg))***
O-acetylserine	0.75 ± 0.09	94.3 ± 22.1
Sodium sulfide	4.02 ± 0.26	86.8 ± 10.2
L-cysteine	0.41 ± 0.03	(2.1 ± 0.5)*10^5
DTT	0.48 ± 0.03	(3.3 ± 1.6)*10^5
D,L-Homocysteine+DTT	no release of hydrogen sulfide detected	
O-succinyl-homoserine+sodium sulfide	no homocysteine synthesis detected	
O-phosphoserine+sodium sulfide	no cysteine synthase activity detected	
D,L-Homocysteine+O-acetylserine	no cystathionine β-synthase activity detected	

Table 1 Kinetic parameters of *L. casei* CysK
Recombinant CysK was assayed for cysteine synthase, cysteine lyase and cystathionine β-synthase activity. The synthesis of cysteine was assayed photometrically as described by Kredich & Tomkins (1966) and confirmed by thin layer chromatography (data not shown). The release of hydrogen sulfide from cysteine was assayed photometrically as described by Fukamachi et al. (2002). Cystathionine synthase activity was assayed as described by Hullo et al. (2007). Assays were performed at 37°C. Values represent the means ±S.D. from three independent experiments.

Strain dependent expression of *cysK*

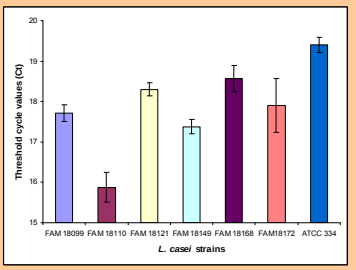

Figure 3 Expression analysis of *cysK* in different *L. casei* strains
Transcript level of *cysK* was determined by real-time PCR and normalized with total RNA amount. Illustrated are the threshold cycle values which are inversely proportional to the original relative expression level. A difference of 3.32 Ct means a difference of 10-fold in copy numbers. Thus, transcript level of *cysK* is approx. 10-fold higher in FAM18110 than in ATCC 334.

Conclusion and Outlook

In this study we showed that *cysK* from *Lactobacillus casei* FAM18110 which is identical to LSEI_0480 from ATCC334 encodes a functional cysteine synthase. The cysK protein synthesized cysteine from O-acetylserine and sodium sulfide and its role in cysteine biosynthesis was confirmed in complementation experiments. Since O-phosphoserine, a constituent of caseins, was not used as substrate and *L. casei* apparently does not possess a gene encoding a serine acetyltransferase, the first step in cysteine biosynthesis still is unclear and needs further investigation. Recombinant CysK also degraded cysteine to hydrogen sulfide in the the presence of a nucleophile. Hydrogen sulfide is found in many cheese varieties and is considered as a key flavor compound. Therefore, *cysK* may play a role in flavor production. Interestingly, we found that the gene is expressed in a strain dependent manner. Currently, we are determining the protein amount of CysK to test the hypothesis that transcript level correlates with protein amount and enzymatic acitivity. This will allow us to clarify the role of *cysK* in aroma formation by using a high and a low *cysK* expressing *L. casei* strain as adjunct cultures in cheese.

Literature

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