Analysis of glutamine synthetase activity from *Lactobacillus hilgardii* LMG 7934

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**Abstract.** Lactic acid bacteria (LAB) are Gram-positive, non-spore forming, facultative anaerobic or microaerophilic bacteria living in various nutrients-rich ecological niches and are widely used for dairy food and silage production as well as probiotics for the Human and animals. While bacteria can assimilate various nitrogen-containing compounds, the glutamine and ammonium are the most preferred nitrogen sources since they could be directly involved into the nitrogen metabolism of the cell. In bacterial cells, the glutamine can be synthetized from glutamate and ammonium ions by the metalloenzyme glutamine synthetase (GS). In contrast to other bacteria which generally have one gene encoding for the glutamine synthetase, two genes encoding proteins with 53% mutual identity and predicted glutamine synthetase activity were found in the genome of *Lactobacillus hilgardii* LMG 7934. One gene (*glnA_2*) is located in the *glnRA* operon with the transcriptional factor GlnR gene (*glnR*) similarly to GS genes from other bacteria. The second GS gene (*glnA_1*) is monocistronic. While the biosynthetic activity glutamine synthetases could be detected in *L. hilgardii* cells, which protein plays the major role is still unclear.

**Keywords:** *Lactobacillus*, nitrogen metabolism, glutamine synthetase

1. Introduction

Lactic acid bacteria are widespread in different ecological niches with the excess of nutrients. *Lactobacilli* are used by humans in the food industry, the production of dairy food and probiotics, lactic acid and silage [1]. Despite wide use, many aspects of the nitrogen metabolism of *Lactobacilli* remain unexplored.

Most bacteria are not capable of biological nitrogen fixation. Microorganisms can use various compounds containing nitrogen in a reduced or oxidized state as nitrogen sources [2]. Nitrogen sources other than ammonia are converted to it using the ATP energy and NADH. Microorganisms prefer nitrogen sources mainly because those substances require the least energy consumption. Thus, the most preferred sources of nitrogen for many bacteria are glutamine and ammonium, which are directly involved in nitrogen metabolism of the cell [3]. Glutamine synthetase (GS) is a metalloenzyme catalyzing the ATP-dependent synthesis of glutamine from glutamate and ammonium ions. This enzyme is part of the GS/GOGAT cycle of ammonium assimilation, which is active in conditions of low concentrations of ammonium ions. Glutamate synthase (GOGAT) synthesizes two glutamic acid molecules from glutamine and 2-oxoglutarate. One molecule is consumed for the cell needs, and the second is converted to glutamine by glutamine synthetase in the presence of ammonium ions [4]. While lactic acid bacteria are widespread in various ecological niches with the excess of nutrients, the presence...
of gene encoding glutamine synthetase in their genome suggests the fundamental role of this enzyme for the cell.

2. Materials and methods
The *Lactobacillus hilgardii* LMG 7934 (ATCC 27305) strain was obtained from the Belgian Coordinated Collections of Microorganisms (BCCM). Bacteria were grown in Man-Rogosa-Sharpe (MRS) Broth (SigmaAldrich, USA) under microaerophilic conditions at 37 °C for 24 – 96 h. To simulate the nitrogen starvation the MRS broth was prepared 1) without yeast extract or 2) both peptone and yeast extract. The genomic DNA was extracted using GeneJET Genomic DNA Purification Kit (ThermoFisher, USA). The quality and purity of DNA was checked by using 0.7% agarose gel electrophoresis, while the quantity was determined using Nanodrop2000 systems. The *glnA* genes were cloned into expression vectors using Gibson’s reaction [5]. The GS proteins were purified using affinity chromatography on Ni-NTA sepharose [6]. The GS enzymatic activity was determined by biosynthetic assay [7].

3. Results and discussion
The GS activity in *L. hilgardii* was measured in cells growing in either full MRS or nitrogen-poor conditions. Thus, the maximum activity has been observed on 24th h of growth in full MRS while no activity was detected in nitrogen-starving cells (Figure 1).

![Figure 1. Glutamine synthetase activity in the *L. hilgardii* cells under different conditions of nutrient availability.](image)

The analysis of *L. hilgardii* genome revealed the presence of two genes for glutamine synthetase [8]. Thus, one gene named as *glnA_2* is located within the *glnRA* operon with the gene of transcriptional factor GlnR (*glnR*). The second gene *glnA_1* is monocistronic. Proteins exhibit 53% mutual identity and 70% mutual homology, while the identity with glutamine synthetases from other bacteria is low (Table 1, 2) suggesting that these genes apparently were not transferred by horizontal transfer from other bacteria.

Both genes were cloned into pET15b expression vector, resulting GS1-his<sub>6</sub> and GS2-his<sub>6</sub> recombinant proteins were purified to an electrophoretic homogeneity by affinity chromatography. The enzymatic activity of GS was measured both in vitro and in vivo. Both purified proteins showed a low level of biosynthetic activity in vitro in comparison with the GS activity in vivo in *Lactobacillus* cell and the question regarding the main enzyme remains still open. Apparently, the recombinant proteins were not properly expressed in *E.coli* cells and the expression should be performed in *Lactobacilli*. 

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Table 1. Identity of $L. \ hilgardii$ glnA_1 и glnA_2 with other glnA gene (%).

|          | glnA_1 | glnA_2 |
|----------|--------|--------|
| B. subtilis (NP_389628.1) | 59     | 65     |
| S. typhimurium (NP_462887.1) | 52     | 53     |
| E. coli (NP_418306.1) | 53     | 53     |

Table 2. Identity of $L. \ hilgardii$ GS1 и GS2 with other GS proteins (%).

|          | GS1 | GS2 |
|----------|-----|-----|
| B. subtilis (NP_389628.1) | 54   | 65   |
| S. typhimurium (NP_462887.1) | 35   | 38   |
| E. coli (NP_418306.1) | 35   | 38   |

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