1. Introduction

*Lactobacillus* are Gram-positive, rod-shaped, facultative aerobic or microaerophilic, non-spore forming bacteria (Makarova et al., 2006). Lactic acid bacteria are widespread in nature and can be found in niches with the excess of nutrients, while could be almost never found neither in soil nor water. Despite extensive use in food industry, in production of probiotics, lactic acid and silage, etc. (De Vos et al., 2009), many aspects of the nitrogen metabolism of *Lactobacilli* remain unexplored. The most preferred nitrogen sources for most bacteria are glutamine and ammonium ions. In bacterial cells an enzyme glutamine synthetase synthesizes glutamine from glutamate and ammonium ion. While *L. hilgardii* LMG 7934 genome carries two genes encoding glutamine synthetases with 55% of mutual identity of amino acid sequences, which protein plays the major role is still unclear. One gene is located within classical *glnRA* operon with the gene of GlnR-like transcriptional regulator, while the second is monocistronic. In this study we report the cloning of both genes encoding glutamine synthetases from *L. hilgardii* LMG 7934.

2. Materials and methods

The *Lactobacillus hilgardii* LMG 7934 (ATCC 27305) strain from Belgian Coordinated Collections of Microorganisms (BCCM) was used in this study. For the DNA extraction a single colony of *L. hilgardii* LMG 7934 was grown in Man-Rogosa-Sharpe (MRS) Broth (SigmaAldrich, USA) under microaerophilic conditions at 37°C overnight. The genomic DNA was extracted using GeneJET Genomic DNA Purification Kit (ThermoFisher, USA). The *glnA* genes was cloned into expression vectors used Gibson`s reaction. The GS proteins were purified using affinity chromatography on Ni-NTA sepharose. The GS enzymatic activity was determined by biosynthetic assay (Patterson and Hespell, 1985; Fedorova et al., 2013).

3. Results and discussion

In *L. hilgardii* genome, two glutamine synthetase genes are present, one is located in the *glnRA* operon with the transcriptional factor GlnR gene (*glnR*), and the second gene is monocistronic. Both genes were cloned onto pET15b expression vector, resulting GS1-his<sub>6</sub> and GS2-his<sub>6</sub> recombinant proteins were purified to an electrophoretic homogeneity. To test the enzymatic activity of GS *in vitro* and *in vivo* assays was performed. Both purified proteins showed a low level
of biosynthetic activity \textit{in vitro} in compare with the GS activity \textit{in vivo} in \textit{Lactobacillus} cells.

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\textbf{References}


