Title: Biochemical characterization of glutamine synthetase homologues in *Paenibacillus sonchi* SBR5

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Abstract:
Glutamine synthetase (GS) is a central enzyme in nitrogen metabolism with an essential role in L-glutamine biosynthesis and nitrogen assimilation to the vast majority of Bacteria. Glutamine synthetase catalyzes the reaction that forms L-glutamine from L-glutamic acid and ammonia, using ATP. The introduction of ammonium into the cellular metabolism is carried out by GS and glutamate synthase, producing L-glutamine and L-glutamate. The genus *Paenibacillus* is object of several studies, yet more analyzes are needed regarding genomics due to its relevance, broad distribution, important function in microbial communities, and nitrogen fixation, noticed in several species of the genus. Thus, they are capable of contributing in distinct fields of agricultural biotechnology. The bacterium *Paenibacillus riograndensis* (SBR5), a heterotypic synonym of *P. sonchi* (X19-5^T), a nitrogen-fixing bacterium isolated from *Triticum aestivum*, presents peculiar characteristics when compared to others of the genus. SBR5 presents three GS homologous coding genes (appointed in the present study as “GSs like”- GSL1, GSL2, and GSL3). This project aims to investigate these genes’ functions. The three proteins (GSL1, GSL2, GSL3) were expressed in *Escherichia coli* BL-21, using pASK-IBA3 plasmid. Analysis of polyacrylamide gel (SDS-PAGE) confirmed their expression. Proteins were purified by Strep-tactin affinity chromatography, using the medium StrepTactin™ Sepharose™ High Performance (GE Healthcare), according to manufacturer’s instructions. After purification, proteins were dialyzed, quantified by the Bradford method, and stored at -20 °C for further analysis. The biosynthetic activity was measured by a microtiter colorimetric assay based on phosphate release from ATP hydrolysis by the GS. The activity was detected only from GSL2. Keeping in mind the research already performed about GSL1, other analyses must be realized to validate its activity, which includes new purification. Regarding GSL3, parallel phylogenetic studies indicate it does not display biosynthetic activity. Besides, proteins modulation will be evaluated by testing potential inhibitors such as glycine, histidine, alanine, p-aminobenzoic acid, arginine, asparagines, and cysteine.

Keywords: nitrogen fixing, nitrogen metabolism, biosynthetic reaction.

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