

**TITLE:** *Paenibacillus sonchi* SBR5 displays three glutamine synthetase homologs with distinct evolutionary histories

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**ABSTRACT:**

Glutamine synthetase (GS) catalyzes glutamine formation from glutamate and ammonium with ATP hydrolysis, representing the central nitrogen assimilation pathway in most organisms. Being encoded by ancient genes, GSs comprise a wide superfamily, divided into three families (types I, II, and III). *Paenibacillus sonchi* SBR5 is a diazotrophic bacterium isolated from wheat rhizosphere in Brazil. Evaluating SBR5 nitrogen metabolism, we noticed its genome harbors three GS-homologous encoding genes. In order to evaluate their origins and relationships, we performed a Bayesian phylogenetic analysis. *Bacillus subtilis* GSI (UniProtKB P12425) was used as bait to search complete Archaeal and Bacterial genomes deposited on IMG (<https://img.jgi.doe.gov/>) via BlastP. To discriminate the three families, type II (*Rhizobium leguminosarum* Q02154) and type III (*Bacteroides fragilis* Q5LGP1) GSs were also added. Sequences were aligned using Muscle on MEGA7, and the resulting alignment was edited on GBlocks Server. The edited alignment was evaluated with Prottest to determine the evolutionary model, which was implemented on Beast 1.8.4 using the “Speciation: Birth-death” tree prior and strict clock. The MCMC chain was run for 50 million generations, and the consensus tree was generated with TreeAnnotator. We could determine that the three SBR5 sequences grouped with the type I GSs, while a few sequences grouped with the representatives of the others. These type II and III-related sequences were excluded, generating our second database. The methods described were implemented again to evaluate the relationships between the type I GSs. Regarding the phylogenetic tree obtained, we could observe that our three sequences of interest do not share a recent origin in common. PRIO\_2126 and PRIO\_3849 grouped with the clades recognized as GSI-alpha and GSI-beta, respectively. These two subgroups were originated in an ancient duplication, and we could see in our results that they also harbor sequences from other *Paenibacillus* genomes. PRIO\_3736, on the other hand, lies apart from any other *Paenibacillus* GS evaluated here. It relates to sequences that do not display GS biosynthetic activity but participate in polyamine catabolism. In conclusion, the three SBR5 GSs are type I-related sequences and do not result from recent duplications. We believe two of them can perform distinct physiological functions, conserved in the genus, and the third one may participate in polyamine degradation.

**Keywords:** gene family, sequence evolution, nitrogen.

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