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**Research Article**

# Cloning and Expression of Glutaminase New Gene in the Sauce Billet Metagenomic

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**Abstract:** Aim to study the Glutaminase gene structure and function the prokaryotic expression vector pEAZY E1-glsA927 is construnted from the new genes glutaminase named glsA927 which is cloned in the traditional sauce billet microbial metagenomic and connected to pEAZY E1 carrier. After the recombinant plasmid is passed into E. coli BL21 and induced by gene expression and after purified by the Ni column, express product detect the enzyme activity by means of bacteria glutaminase activity quantitative detection kit. Sequence analysis results show that the gene length of glsA927 is 927 bp, which has the highest sequence homology (AF057158.1), 94.82%, reported on GENBANK, with 16 different amino acids. Protein expression analysis results show that when the concentration of IPTG tendency is 0.2 mmol/L, protein expression of induced 4 h has the highest amount. The Ni ion affinity chromatography purification of recombinant glutaminase ihs-glsA927, preliminarily determine glutaminase activity to a maximum of 468.7 U mu/g, SDS-PAG and Western results show that recombinant glutaminase His-glsA927 which is purified by the Ni ion affinity chromatography could be preliminarily determined that the glutaminase activity can reach as much as 468.7 U/μg and not easy to be affected by the concentration of NaCl.

**Keywords:** Clone, glutaminase, metagenome, sauce bille

## INTRODUCTION

Glutaminase (EC 3.5.1.2), with gamma pancreatic acyl transfer ability and glutamine hydrolysis activity, which can hydrolyze L-glutamine into L-glutamic acid and ammonia, is a key enzyme and rate-limiting enzyme which is decomposed by the glutamine in the living body (Hu *et al*., 2010; Suzuki *et al*., 2010). At the same time, Glutaminase (EC 3.5.1.2) also has the vital significance in the production of condiments with the soybean as raw material (Huang *et al*., 2014; Liu *et al*., 2011). Soybeans almost half of the total protein amino acid composition of exists in the form of glutamine, therefore glutaminase activity is to determine major umami substances content in soybean fermentation food one of the important factors (Han *et al*., 2001; Nandakumar *et al*., 2003). About half of the glutamic acid of total protein amino acid composition in the soybeans exits in the form of glutamine, therefore glutaminase activity is the leading factor in determining the content of major umami substances in the soybean fermentation food. The cloned glsA2 gene with high glutaminase activity from bacillus subtilis strains and tested that the glutaminase activity of the encoding gene is up to 608.2 U/mg through the prokaryotic expression (Lu *et al*., 2013; Zhan *et al*., 2011). Kumar *et al*. (2012) successfully cloned glutaminase encoding gene with tolerate of high PH value and high salt concentration.

Based on the ability of screening and cloning of glutaminase gene of cultured strains, it makes many good genes of strains which are difficult to culture is likely to be missed. Microbial community in a particular environment is the main research object in metagenomic technology macro genomics. Therefore, targeted good genes with clear expectation characteristics can be acquired by cloning. For example, Zhang *et al*. (2013) cloned the beta galactose glucoside enzyme which is from the turpan basin soil metagenomic library, China. It has the characteristic of high temperature resistance (the optimum temperature is 78°C). Wang *et al*. (2014) cloned EPSPS gene of high tolerate levels of glyphosate from the soil metagenomic clone contaminated by glyphosate by high pressure filtration.

In this study, glutaminase new genes, glsA927, is cloned from traditional sauce billet microbial metagenomics with the PCR technology, through which the pEAZY E1-glsA927 prokaryotic expression vector is built. The restructuring of glutaminase His-glsA927 prokaryotic expression is realized by IPTG induction and preliminary determined the enzyme activity in different conditions, which laid a solid foundation of

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further utilization of the enzyme for deep processing of soybean industry.

## THE EXPERIMENT MATERIALS

**Strains and plasmid:** Traditional sauce billet microbial compound bacteria is bought from the farmers in Changchun city, Jilin province; the escherichia coli cells Trans1-T1, BL21 and the prokaryotic expression vector pEASY-E1 Expression Kit are purchased from Quan Shijin biotechnology co., LTD, Beijing.

**Enzymes and kit, biochemical reagents:** Bacterial genome extraction kit enzyme is bought from Tiangen biochemical science and technology (Beijing) co., LTD; EasyPfu DNA Polymerase enzymes are from Quan Shijin biotechnology co., LTD, Beijing; plasmid small extraction kit from Zi Bao biological engineering (Dalian) co., LTD.; Other chemicals such as ethanol, chloroform, phenol etc. are analytical reagent of traditional Chinese medicine; Bacterium Glutaminase Assay Kit from the Genmed company, the United States.

## EXPERIMENTAL METHODS

**The building of cloning and expression vector of glutaminase new gene glsA927:** Melt the traditional sauce billet bought from the farmers with isopyknic sterile water, put the filtrate which is filtrated after 4 layer gauze filter in the centrifuge tube and centrifuge with 12000 RPM for 10 min to collect the microorganisms, extract the total DNA with the Bacterial genome extraction kit, design acquire primers glsAF (5 '-ATGGCGGATTTGCAgycgrccntc -3') and glsAR (5 '-TTAATGCCGAAAAccnacragyc -3'), according to the glutaminase gene (JF521500.1, AF057158.1) which is published on GENBANK using the CODEHOP online tools (http://blocks.fhcrc.org/codehop.html) with the template of TE which is diluted to 50 ng/μL and then amplify the purpose fragment with the PCR method. The procedure of PCR is: pre modified 4 min under 95°C; modified 30 SEC under 95°C; annealing 30 SEC under 54°C; extend 1min under 72°C; circulate the procedure for 30 times; and finally extend 5 min under 72°C.

PCR products directly connected to the expression vector pEASY-E1 in accordance with the pEASY-Kit instructions. Screen the blue white spot after transforming the escherichia coli competent cells Trans1 and then the restructuring ones are sequenced by the Shanghai jerry gene co., LTD. And the sequence is bioinformatic analyzed by the software of Blast, DNAman etc.

Insert glsA927 gene in the right direction of clone, named pEASY no.e1 -glsA927. Extract the plasmid transformation engineering bacterium BL21 competent cells.

**Expression and purification of glsA927 gene:** When engineering bacterium BL21 cells carrying a prokaryotic expression vector pEASY no.e1 -glsA927 is cultured in LB medium under 37°C to OD value of 0.6, add 0, 0.1, 0.2, 0.3, 0.4, 0.5 mmol/L IPTG, respectively and analyze the difference of the induced results under different IPTG concentration by SDS electrophoresis. And induce 0, 2, 4, 6, 8 h after joining the IPTG of 0.2 mmol/L, then analyze the differences of the results of different induction time.

Centrifugally collect the bacteria from the 4hr bacteria liquid which was induced by 0.2 mmol/L IPTG and resuspend the bacteria with the PBS buffer of 0.1 times of the volume, collect the supernatant after breaking by the ultrasonic through 15000 g centrifugal for 5 min and then purify the interest protein with the Ni column. Analyze the purification effect with the SDS electrophoresis and Western hybridization with eluent of the imidazole of 10, 20, 40, 100, 200, 300, mmol/L in proper order.

**Glutaminase activity analysis:** Test the enzyme activity which is under the purification of objective protein by the Ni column using the bacteria glutaminase activity quantitative test kit. Determine the activity of restructuring of enzyme under the temperature of 4, 25, 37, 42, 50, 65 and 70°C, respectively successively, to analyze the influence of temperature on their activity; Under the condition of 50°C, NaC1 is added to the reaction system, with the final concentration of 0, 5, 10, 5, 20, 25, 20%, respectively, to determine the enzyme activity of recombinant enzymes under different salt concentration.

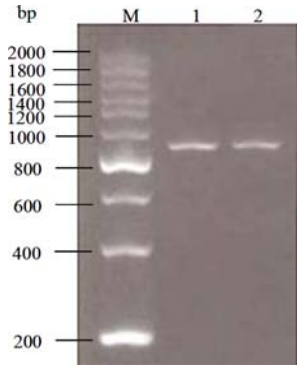
Glutaminase unit activity is defined as: a unit of vitality is the enzyme amount which is needed when 1 μmol glutamine is switched to glutamic acid in 1 min.

## RESULTS

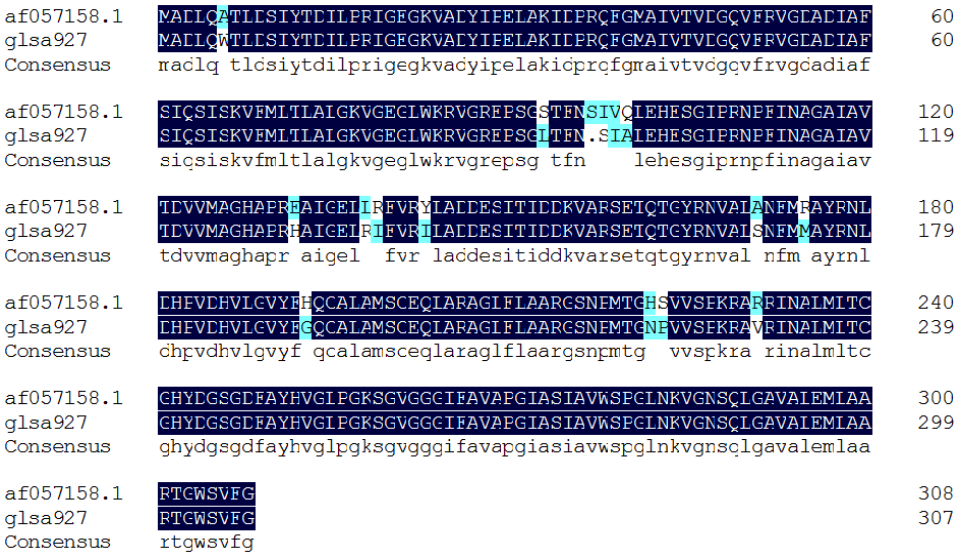
**The construction of glsA927 gene cloning and prokaryotic expression vector:** As what is shown in Fig. 1a -A, DNA fragments of the size about 0.9 kb is successfully obtained by PCR.

Connecting the clip to the prokaryotic expression vector pEASY E1 and sequencing, it is found that the gene 927 bp, the coding sequence of amino acids, has the highest homology with the bean rhizobia glutaminase AAC63991.1, published on GenBank, which is 94.82%, with a total of 16 amino acid differences (Fig. 1b). The gene is named as glsA927 and select the recombinant as shown in Fig. 1c, namely, the T7 promoter-glsA927 gene-T7 recombinant of terminator, named pEASYE1-glsA927.

**The expression and purification of glsA927 gene:** By comparing different induction conditions, it is found that, when adding IPTG with the concentration of 0.2 $L^{-1}$, the protein expression of the target gene quantity no longer increases (Fig. 2a); And after the induction time



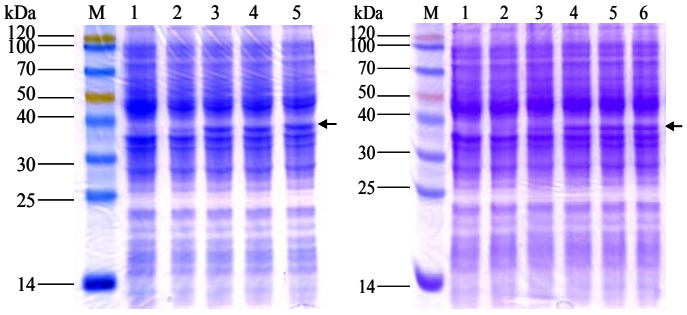

(a)




(b)


(c)

Fig. 1: The construction of cloning and prokaryotic expression vector of glsA927 gene




(a) (b)

Fig. 2: The expression of his-glsA92 detected with SDS-PAGE; A: The effect of IPTG concentration on the expression, M: Blue Plus Protein Marker, 1-6: With 0, 0.1, 0.2, 0.3, 0.4, 0.5 mmol/L IPTG; B: The effect of time on the expression, M: Blue Plus Protein Marker, 1-5: 0, 2, 4, 6, 8 h

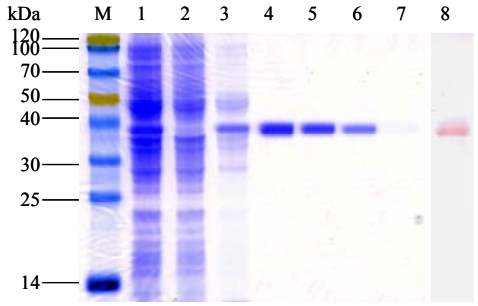



Fig. 3: Purification and identification of the fusion protein his-glsA92; M: Blue plus protein marker, 1: The sample; 2: flow; 3-7: Eluent of imidazole containing 40, 80, 120, 160, and 200 mmol/L; 8: Western hybridization of the eluent of 200 mmol/L

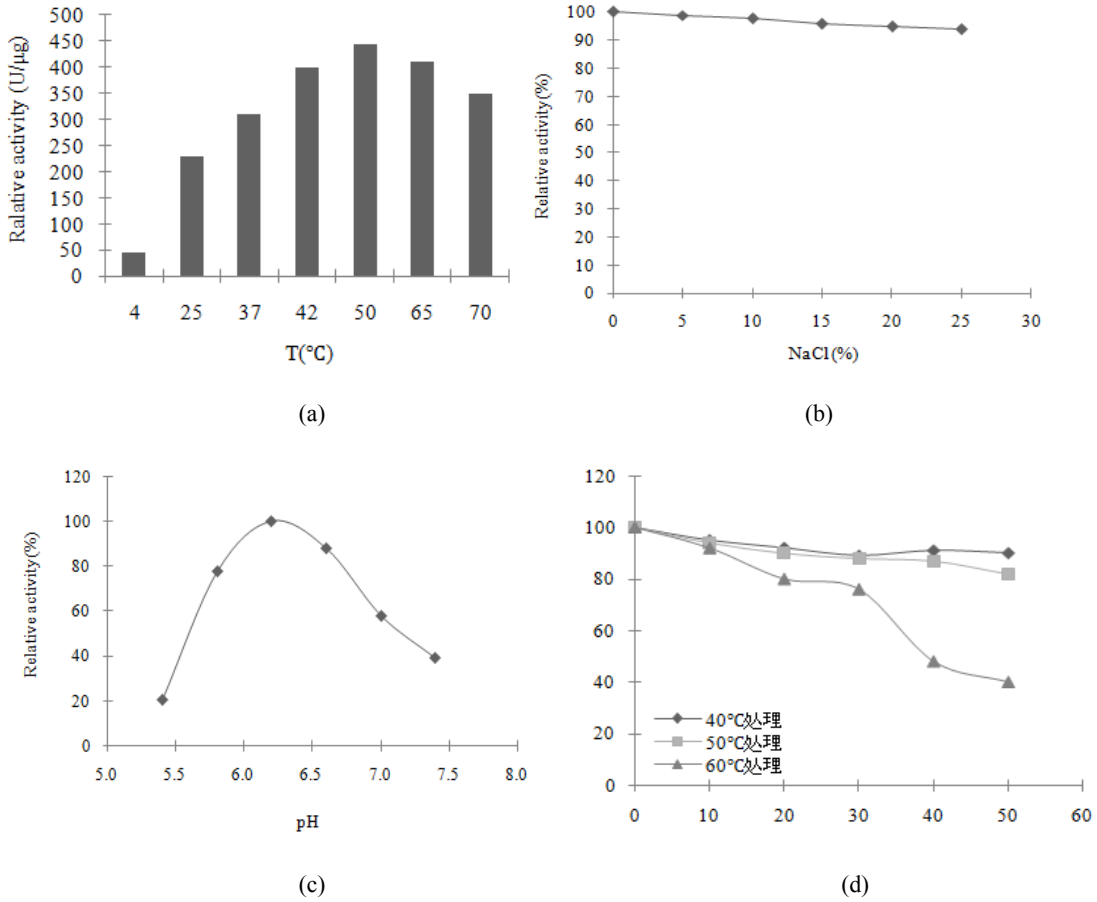



Fig. 4: The influence of different processing on the enzyme activity; a: The influence of temperature on the enzyme activity; b: The influence of NaCl on enzyme activity; c: The influence of Ph on enzyme activity; d: Stability analysis

reaching 4 h, the amount of the protein expression of target gene also has no significant changes (Fig. 2b). It can be concluded that induced by the IPTG of 0.2 mmol/L for 4 h under 28°C is the best inducing conditions. As is shown in Fig. 3, the hybridization signal of recombinant proteins has been found in the eluent of imidazole of 40 mmol/L, but there is still a complex protein; a lot of the target protein is washed-out in the eluent with the concentration of imidazole more than 80 mmol/L and the purity can satisfy the further demands of the experiment; the amount of the target protein in the eluent with the concentration of 200 mmol/L is few and only can be detected by western hybridization.

**Analysis of glutaminase activity:** Under different conditions, the test results of enzyme activity can be found that when the temperature is 50°C, glutaminase enzyme activity of gene encoding glsA927 reached the maximum, 468.7 U/μg. The active influence of NaCl is not obvious. When the concentration of NaCl is close to the saturated concentration (25%), this enzyme remains

in the activity of 419.3 U/μg, which is about 89.1% of the vitality without NaCl (Fig. 4).

## DISCUSSION AND CONCLUSION

The experimental results show that the new glutaminase gene glsA927, which is got from the sauce billet microbial metagenomics, is cloned by the merger primers designed by the CODEHOP method. The amino acid sequence of this gene encoding and the known bean rhizobia glutaminase (AAC63991.1) have the highest homology, only 11 different amino acid. Construct prokaryotic expression vector pEAZY no.e1-glsA927, after IPTG induced genes and Ni column were obtained by ihs-glsA927 by 468.7 U in optimal conditions were measured muon/g and enzyme activity. Affected by the, for engineering enzyme for. After induced by IPTG gene and purified by Ni column, the construction of prokaryotic expression vector pEAZY no.e1-glsA927 can obtain restructuring glutaminase His-glsA927 which is measured to reach 468.7 U/μg under the optimal conditions and the enzyme activity affected by the concentration of NaCl is not obvious. It is the suitable engineering enzyme for industrial production of soybean fermentation in the production of lobster sauce, soy sauce and other non-staple food.

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