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Purification and characterization of extracellular glutaminase from *Aspergillus oryzae* NRRL 32567

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ABSTRACT

The glutaminase produced from *Aspergillus oryzae* NRRL 32567 was purified (10.2 folds) using ammonium sulfate fractionation followed by gel filtration on Sephadex G-75. SDS-PAGE of the purified glutaminase showed the presence of one band with a molecular weight of 68 kDa. Optimum pH was 7.0 while a temperature range 30–40 °C was optimal the activity. The highest pH stability was obtained at pH 7.0 while a temperature range 30–40 °C resulted in the highest temperature stability. The apparent K_m value was calculated from the Linenweaver-Burk plot and was found to be 4.5 mM. Glutamine was the preferred substrate for the enzyme and the maximum relative activity of 130% was observed at 2.5% glutamine . Potassium showed a slight increase in activity of glutaminase especially at the concentration of 0.5 mM. While, frerric ions followed by ferrous showed the highest inhibition effect on glutaminase.

1. Introduction

Glutaminase (EC 3.5.1.2) is an amidohydrolase enzyme which generates L-glutamic acid and ammonia from L-glutamine. A variety of microorganisms, including bacteria, yeasts and molds have been reported to produce L-glutaminase (Kashyap et al., 2002; Weingand-Ziadé et al., 2003; Iyer and Singhal, 2008) of which the most potent producers are molds (Balagurunathan et al., 2010). On an industrial scale, glutaminases are mainly produced by the genera Aspergillus and Trichoderma (Yamamoto and Hirooka, 1974; El-Sayed, 2009). *Aspergillus oryzae* is a filamentous fungus, which has an ability to secrete large amounts of hydrolytic enzymes. It is widely used in the manufacture of traditional fermented soy sauce in Asia (Wood, 1977). Moreover, *A. oryzae* is genomically well characterized and considered to be a safe organism for producing of food enzymes because it lacks expressed sequence tags for the genes responsible for aflatoxin production (Fabio et al., 2012). Also, it is widely recognized as preferable L-glutaminase sources because they generally produce extracellular enzymes, which facilitate the enzyme recovery from the fermentation broth (Koibuchi et al., 2000).

In recent years, L-glutaminase has attracted much attention in both pharmaceutical and food industrial applications. In food industry, L-glutaminase is used for the production of glutamic acid, which is the most important amino acid in food manufacture for a delicious taste (O`Mahony and Ishi, 1987; Fukushima and Motai, 1990). The pleasant and palatable tastes of oriental fermented food like soy sauce, miso and sufu are considered to be related to their content of L-glutamic acid accumulated due to the hydrolysis of a protein catalysed by proteolytic enzymes, including L-glutaminase, protease and peptidases (Lu et al., 1996). Salt tolerant L-glutaminases are most valuable in the industrial processes that require high salt environments like the soy sauce fermentation. L-glutaminases from conventional sources (*Aspergillus oryzae*) are markedly inhibited by high salt concentrations as demonstrated by Kumar et al. (2012). Salt tolerant L-glutaminases were patented for use in industrial processes (Sabu et al., 2000). Tumor growth regulation can be achieved by inhibition of both protein and nucleic acid biosynthesis in the cancerous cells due to the lack of availability of any component of these macromolecules. Inhibition of the tumor cell uptake of glutamine is one of the possible ways to stop the growth and this is the best accomplished by the use of L-glutaminase, which breaks down L-glutamine. This in fact, results in a selective starvation of the tumor cells because unlike normal cells they lack properly functioning glutamine biosynthetic machinery (Tanaka et al., 1988). Another important application of L-glutaminase is in biosensors for monitoring the glutamine levels in mammalian and hybridoma cells (Kashyap et al., 2002; Klein et al.,

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2002). The objective of this work was focused on the purification and characterization of glutaminase from *Aspergillus oryzae* NRRL 32567.

2. Materials and methods

2.1. Enzyme

The crude L-glutaminase was prepared from *Aspergillus oryzae* NRRL 32567 (as a new source for glutaminase) as early described by Alian et al. (2015) as following: Fifty milliliters of a medium containing ($g\,L^{-1}$): yeast extract, 20; lactose, 25; glutamine, 5; KH_2PO_4, 1.5; K_2HPO_4, 3; NaCl, 5 and Mg $SO_4 \cdot 7H_2O$, 5 at pH 7.0 were placed in 250 ml Erlenmeyer flasks and autoclaved at 121 °C for 15 min. Each of the sterilized flasks was inoculated with 1 ml spores suspension (1.5×10^7 spores ml^{-1}), placed in a rotary shaker (100 rpm) and the growth was aerobically carried out at 30 °C for 3 days. At the end of the incubation period, the mycelia were recovered from each flask by filtration on Whatman no. 1 (Whatman Ltd., Maidstone, England) and culture filtrate was used as the source of crude glutaminase.

2.2. Assay of L-glutaminase

L-glutaminase activity was determined using the method of Imada et al. (1973) and as modified by Alian et al. (2015) utilizing L-glutamine as substrate and the released ammonia was measured using Nesseler's reagent. An aliquot of 0.5 ml of the sample was mixed with 0.5 ml of 0.04 M L-glutamine solution in the presence of 0.5 ml of distilled water and 0.5 ml phosphate buffer (0.1 M, pH 7.0). Then the mixture was incubated at 37 °C for 30 min and the reaction was stopped by the addition of 0.5 ml of 1.5 M Trichloroacetic acid. To 0.1 ml of the mixture, 3.7 ml of distilled water and 0.2 ml of Nessler's reagent were added. The absorbance was measured at 430 nm using a spectrophotometer, Model 6300 (Jan way LTD., Essex, U.K). A standard graph was plotted using ammonium chloride. One international unit of L-glutaminase was defined as the amount of enzyme that liberates one µmol of ammonia under optimum conditions. One international unit of L-glutaminase was defined as the amount of enzyme that liberates one µmol of ammonia under optimum conditions.

2.3. Determination of enzyme protein

Enzyme protein was spectrophotometrically determined according to the method of Lowry et al. (1951) using Bovine Serum Albumin as a standard.

2.4. Glutaminase purification

2.4.1. Fractionation by ammonium sulfate

Different saturation levels of ammonium sulfate were used (20%, 30%, 40%, 50%, 60%, 70%, 80% and 90%). Known volumes of crude enzyme filtrate were successively treated with such levels of ammonium sulfate for overnight at 5 °C and then centrifuged at 8000 rpm for 20 min (Abdallah et al., 2013). The obtained precipitate was overnight dialyzed in a cellulose bag (Fisher Scientific Company. USA) against sodium phosphate buffer (0.1 M, pH 7.0) at 5 °C under mild agitation. The buffer was changed each 4 h. An aliquot was then collected and analyzed for glutaminase activity and protein content.

2.4.2. Gel filtration chromatography

The dialyzed enzyme solution (1 ml) was added to a Sephadex G-75 column (1.0×45 cm) previously equilibrated with sodium phosphate buffer (pH 7.0, 0.1 M) and eluted with the same buffer without change in ionic strength at a flow rate of 0.6 ml min^{-1}. Fractions of 3.0 ml were collected and both protein content and glutaminase activity were off-line determined. Protein content in the elutent was spectrophotometrically measured at 280 nm (Ali et al., 2009).

2.4.3. Gel electrophoresis (SDS-PAGE)

The purity of L- glutaminase protein was tested by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) using coomassie brilliant blue dye according to the procedure of Ali et al. (2009).

2.5. Characteristics of the purified glutaminase

2.5.1. Optimum reaction temperature

The effect of temperature on purified glutaminase activity was studied by carrying out the enzyme reaction for 30 min at different temperatures (25, 30, 40, 50, 60 and 70 °C) using the same enzyme assay conditions previously described.

2.5.2. Thermal stability

The purified enzyme was incubated at different temperatures (30, 40, 50, 60 and 70 °C) for various lengths of time up to 2.0 h. After cooling, the residual activities were determined under the optimized assay conditions.

2.5.3. Optimum reaction pH

The effect of pH on purified enzyme was determined by carrying out enzyme reaction at different pH levels using different buffer systems (0.1 M): sodium acetate buffer (pH 3.0–5.0), sodium phosphate buffer (pH 6.0–8.0) and boric acid borate (pH 9.0–10) using the same enzyme assay conditions previously described.

2.5.4. pH stability

The purified enzyme was incubated at various pH levels (3.0–10). Such levels were achieved by the application of different buffer systems (0.1 M): sodium acetate buffer (pH 3.0–5.0), sodium phosphate buffer (pH 6.0–8.0) and boric acid borate (pH 9.0–10). Samples were withdrawn at time intervals and the enzyme activity was assayed under the optimized assay condition.

2.5.5. K_m determination

The Michaelis constant (K_m) of the purified glutaminase was determined by measuring the reaction velocities (µM ammonia min^{-1}) at various concentrations of glutamine (mM ml^{-1}) at 30 °C for 30 min. the data were plotted according to Lineweaver and Burk (1934).

2.5.6. Substrate specificity

Different substrates i.e., L-glutamine, D- glutamine, L- asparagine, D- asparagine, L- glutamate (monosodium glutamate) and L-glutamic acid were used. Each one was separately added to the reaction mixture in amount of 0.04 M and glutaminase activity was measured in normal conditions and the relative activity was calculated.

2.5.7. Salt-tolerant

The activity of purified enzyme was assayed in the standard reaction mixture supplemented with NaCl at various concentrations (0–20%, w/v) and the relative activity was calculated.

2.5.8. Effect of various metal ions

Metal ions such as: K^+, Na^+, Cu^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Hg^{2+} and Zn^{2+} were separately tested for their effect on glutaminase activity. Salts of the different metals were supplemented

with reaction mixture at concentrations of 0.1, 0.2 and 0.5 mM for each. Glutaminase activity was determined and the residual activity was measured.

2.6. Statistical analysis

Results were subjected to one-way analysis of variance (ANOVA) (Rao and Blane, 1985). All results were the average of three experiments.

3. Results and discussion

3.1. L-glutaminase purification

3.1.1. Ammonium sulfate fractionation

Results indicate the maximum specific activity of 30.49 U mg^{-1} was obtained at 70% ammonium sulfate saturation followed by 24.18 and 22.39 U mg^{-1} at 60% and 50% saturation, respectively. At such levels the increase in specific activities was 6.1, 4.8 and 4.5, respectively.

Jayabalan et al. (2011) used 60% ammonium sulfate saturation to precipitate a glutaminase from *Brevundimonas diminuta* MTCC 8486. At such level of ammonium sulfate, the specific activity and the degree of purification reached 21.9 U mg^{-1} and 17.5 respectively. Abdallah et al. (2013) showed that at 80% saturation, the maximum values of total activity (122.3 U ml^{-1}), specific activity (9.7 U mg^{-1}) and yield (6.6%) of the L-glutaminase from *Streptomyces avermitilis* were obtained. They also, indicated a purification fold of 2.0 at the same saturation level. The previous findings were identical to that reported by Balagurunathan et al. (2010) who reported a saturation level of ammonium sulfate of 80% to obtain the highest yield of L-glutaminase activity from *Streptomyces olivochromogenes*.

3.1.2. Gel filtration chromatography

The precipitated protein obtained after treatment of the crude enzyme extract with ammonium sulfate (70% saturation) was dissolved in sodium phosphate buffer (pH 7.0, 0.1 M) and dialyzed (with gentle stirring) in cold sodium phosphate buffer for 24 h with 4 changing of the buffer. After dialysis, the enzyme solution (1 ml containing 13.3 mg protein and with a total activity of 405.6 units) was applied to Sephadex G- 75 column (1 × 45 cm) and protein was eluted with the same buffer. Absorbance at 280 nm indicated four peaks (Fig. 1) at fractions10–13,14–24, 57–60 and 61–64. Enzyme activity determination showed high glutaminase activity at peak no. 2 (fractions14–24) only.

The purification of glutaminase is summarised in Table 1. The use of 70% ammonium sulfate saturation resulted in an increase of 6.10 folds in purification. While, the additional use of gel filtration purification step increased purification by 4.1 times above that obtained with ammonium sulfate alone, with recovery of 2.4% of glutaminase activity.

Kumar et al. (2012) reported that, enzyme has been purified 49 fold from cell-free extract with 25% recovery (specific activity 584.2 U mg^{-1} protein) using $(NH_4)_2SO_4$ (30% saturation) followed by anion exchange chromatography and gel filtration. Abdallah et al. (2013) purified glutaminase from *Streptomyces avermitilis* using ammonium sulfate fractionation, followed by DEAE cellulose column. They obtained 8.02 fold of purification.

3.1.3. Electrophoresis

SDS-PAGE of the pooled sample from the gel filtration step shows the presence of one band with the molecular weight of 68 kDa (Fig. 2).

In agreement with this result, Kumar et al. (2012) reported 66 kDa as the molecular weight of purified glutaminase from

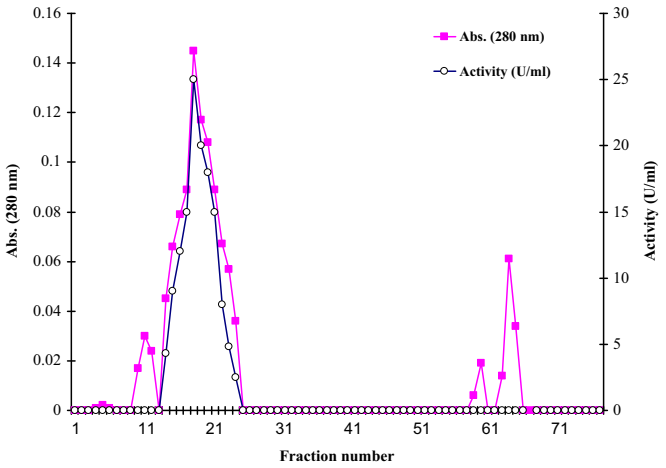

Fig. 1. Fractionation of ammonium sulfate precipitated glutaminase on Sephadex G-75 column (1.0 × 45 cm). Flow rate = 0.6 ml/min. Elution with sodium phosphate buffer (pH 7.0, 0.1 M). Fraction volume = 3 ml.

Table 1
Purification of glutaminase from *Aspergillus oryzae* NRRL 32657.

Purification step	Total activity (units)	Total protein (mg)	Specific activity (U mg^{-1})	Purification (fold)	Yield (%)
Crude enzyme	4653	932	4.99	1.0	100
$(NH_4)_2SO_4$(70%)	405.6	13.3	30.49	6.1	8.70
Gel filtration	112.2	2.20	51.02	10.2	2.40

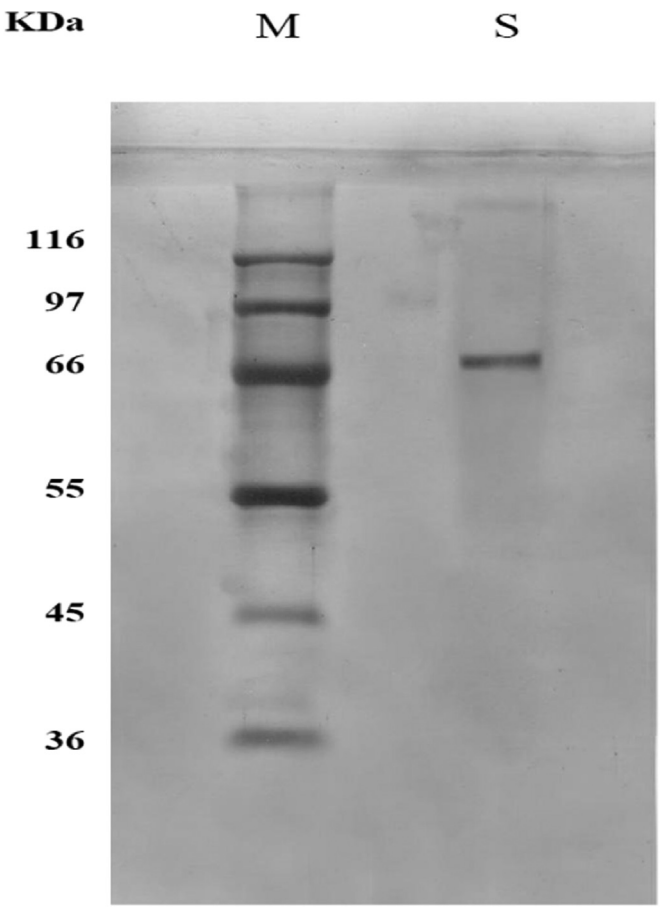

Fig. 2. SDS-PAGE of glutaminase produced by *Aspergillus oryzae* NRRL 32657, where S = Sample and M = Molecular markers.

Bacillus sp. LKG-01 (MTCC 10401). L-glutaminase with 55 kDa was produced by *Bacillus subtilis* (Kozlov et al., 1972), whereas the molecular weight of same enzyme from fungal strains *Aspergillus oryzae* MA-27-IM and *A. oryzae* AJ11728 was found to be 113 and 82 kDa by Ogasawara et al (1996); Klein et al. (2002), respectively. Wakayama et al. (1996) found that the molecular weight of purified glutaminase from *Micrococcus luteus* K-3 was 48 kDa. On the other hand, L-glutaminase with molecular mass of 132 and 137 kDa was reported for *Acinetobacter glutaminasificans* and *Pseudomonas aeruginosa*, respectively (Nandakumar et al., 2003). Previous studies have found that the molecular weight of the purified glutaminase obtained from *Brevundimonas diminuta* MTCC 8486 and *Bacillus cereus* MTCC 1305 was 140 kDa for each (Jayabalan et al., 2011; Singh and Banik, 2013). The purification of L-glutaminase from *Lactobacillus reuteri* by DEAE chromatography was described by Jeong et al. (2010) who determined its molecular weight as 70 kDa. Also, the purified enzyme from *Streptomyces avermitilis* showed a single band on SDS-PAGE and the molecular weight was estimated to be 50 kDa (Abdallah et al., 2013). This variation of molecular weight of L-glutaminases from different sources suggested that the molecular weight of the L-glutaminase is organism specific.

3.2. Characterization of the glutaminase

3.2.1. Optimum reaction temperature

The relation between purified L-glutaminase relative activity and reaction temperature (25–70 °C) was carried out. At 25 °C, only 40% of the maximum activity was achieved. Maximum relative activity (100%) was achieved at 30 and 40 °C, after which a gradual decrease in enzyme relative activity was noticed by increasing the reaction temperature and almost 95% of the activity was lost at 70 °C.

L-glutaminases from bacterial sources showed optimal activities at temperatures above 40 °C. L-glutaminase from *Pseudomonas aeruginosa* and *Micrococcus luteus* exhibited optimum temperature at 50 °C, for both (Soda et al., 1972; Ohshima et al., 1976; Moriguchi et al., 1994). On the other hand, the L-glutaminase from *Cryptococcus albidus* ATCC 20293 showed maximum activity at 70 °C (Iwasa et al., 1987).

3.2.2. Thermal stability

The residual activity of glutaminase was determined at different temperatures for various length of time and the relative activity was calculated (Fig. 3). At 30 °C, glutaminase was very stable without loosing its activity after 120 min, meanwhile it lost 5% of its activity at 40 °C. Data disclose also that, by increasing the incubation temperature the loss of activity was faster and in shorter incubation time. Almost 100% of glutaminase activity was lost after 75 and 45 min at 60 and 70 °C, respectively. At 50 °C about 36% of L-glutaminase activity was retained after 75 min of heating. L-glutaminase obtained from *Aspergillus oryzae* revealed maximum stability at 30–40 °C.

In comparison, L-glutaminase from *Aspergillus oryzae* was stable up to 45 °C and lost its activity completely at 55 °C (Koibuchi et al., 2000). Singh and Banik (2013) found that purified glutaminase from *Bacillus cereus* MTCC 1305 retained 50% and 20% of its stability at 50 and 55 °C, respectively, for 30 min. Nathiya et al. (2011) performed the *Escherichia coli* L-glutaminase stability studies at low temperatures and the authors observed that the exposure of enzyme to cold temperatures resulted in a reversible inactivation of enzymatic activity, while subsequent warming to 24 °C restored the activity and no protein denaturation occurred during this process.

3.2.3. Optimum reaction pH

The effect of the pH level (3–10) of the reaction mixture on the activity of L-glutaminase was studied. Results reveal a gradual increase in enzyme relative activity by increasing the reaction pH and the maximum relative activity (100%) was obtained at pH 7.0. followed by a gradual decrease reaching the lowest values (9%) at pH 10.

Durá et al. (2002) reported pH of 8.5 as the optimum pH for the L-glutaminase from the *Debaryomyces* sp. Whereas Iwasa et al. (1987) reported pH 6.0 for that in *Cryptococcus albidus*.

3.2.4. pH stability

Results (Fig. 4) reveal that, the enzyme exhibited the highest stability at pH 7, followed in descending order by pH 6 and 5. The enzyme retained 100, 75% and 65% of its activity after 75 min of incubation at pH 7, 6 and 5, consecutively. A gradual decrease in activity was noted in case of using pH levels of 4, 8 and 9 reaching 35%, 40% and 55% after 120 min. of incubation. The lowest stability was obtained with pH levels of 3 and 10, where 35% and 100% loss of activity were noticed after 75 and 120 min, respectively.

In agreement with our results, Ohshima et al. (1976) reported the highest glutaminase stability at pH 7.0. In this concern, pH 7.5 was recommended by Roberts et al. (1970) to achieve the highest stability of L-glutaminase from *Pseudomonas aeruginosa*. L-glutaminase from *Debaryomyces* sp. CECT 11815 and *Lactobacillus rhamnosus* showed highest pH stability at pH of 8.5 and 7.0, respectively (Durá et al., 2002; Weingand-Ziadé et al., 2003).

3.2.5. Enzyme K_m

Glutaminase activity increased with increasing L- glutamine concentration, reaching its maximum velocity (V_{max} = 95 μmol^{-1} min^{-1} ml^{-1}) at 50 mM. Huerta-Saquero et al. (2004)

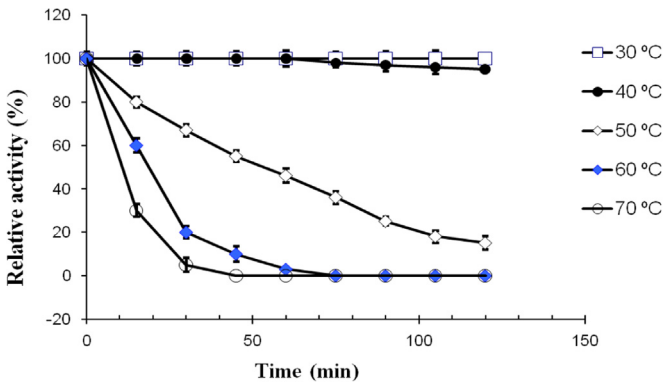

Fig. 3. Thermal stability of glutaminase activity from *Aspergillus oryzae* NRRL 32657.

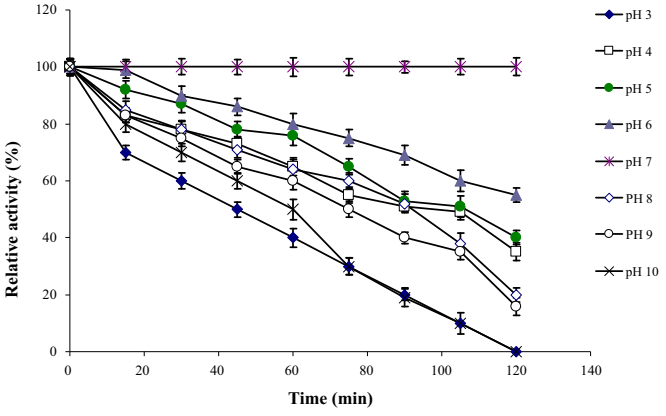

Fig. 4. pH stability of glutaminase activity from *Aspergillus oryzae* NRRL 32657.

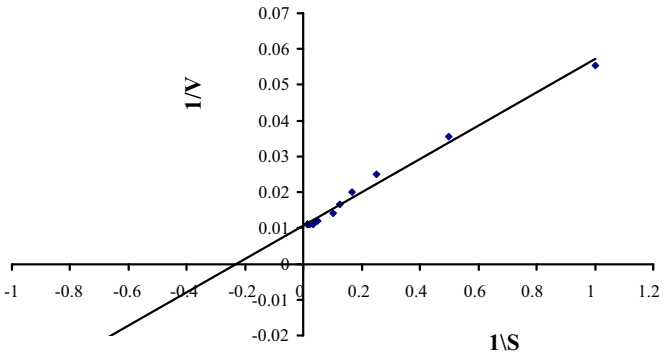

Fig. 5. Lineweaver-Burk Plot for glutamine hydrolysis by glutaminase from *Aspergillus oryzae* NRRL 32657 .

reported 80 mM as the optimal glutamine concentration for maximum glutaminase activity from *Rhizobium etli*. Jayabalan et al. (2011) reported that, the maximum activity of glutaminase from *Brevundimonas diminuta* MTCC 8486 was obtained at 1% of glutamine. The K_m value of L-glutaminase for L-glutamine was found to be 4.5 mM (Fig. 5).

This result indicates the high affinity of L- glutaminase towards its natural substrate. In agreement with this result, Durá et al. (2002) reported K_m value of 4.5 mM for L-glutaminase purified from *Debaryomyces* spp. CECT 11,815. Weingand-Ziadé et al. (2003) reported that the purified L-glutaminase from *Lactobacillus rhamnosus* showed typical Michaelis–Menten behavior with an affinity constant of 4.8 mM for L-glutamine. Singh and Banik (2013) found that K_m and V_{max} of purified glutaminase from *Bacillus cereus* MTCC 1305 were found to be 6.25 mM and 100 μ mol min^{-1} ml^{-1}, respectively. The K_m value was determined as 1.66 mM for L-glutaminase from *Penicillium brevicompactum* NRC 829 (Elshafei et al., 2014). The K_m and V_{max} values were determined as 0.49 mM and 13.86 U L^{-1} for L-glutaminase from *Hypocrea jecorina*, respectively (Bölbül and Karakuş, 2014).

3.2.6. Substrate specificity

The specificity of the glutaminase for several possible substrates was studied. L- glutamine was found to be the favorable substrate with the highest reaction rate (100% relative activity). In addition, D-glutamine, L and D-asparagine were also hydrolyzed at lower rates (23%, 18% and 10% relative activity). On the other hand, the enzyme didn't show activity towards both of L-glutamate and L-glutamic acid which were the products of glutaminase catalytic activity. This could be due to the action of glutamine as an inducer. Such substrate specificity was reported in literature as species related. Kozlov et al. (1972) reported that glutaminase produced from the *Bacillus subtilis* had the capability to hydrolyze the L and D glutamines and lack the L-asparaginase activity. Whereas *Bacillus pasteurii* and *Acinetobacter glutaminasificans* L-glutaminases were able to hydrolyze L-asparagine with low efficiency (Nandakumar et al., 2003). It was reported that L-glutaminase from *Saccharomyces cerevisiae* and *Debaryomyces sp* acted only on L-glutamine (Penninckx and Jaspers, 1985; Durá et al., 2002). Glutaminase from *Bacillus cereus* MTCC1305 showed its specificity for glutamine (Singh and Banik, 2013).

3.2.7. NaCl tolerant

The salt tolerance of glutaminase obtained from the *Aspergillus oryzae* NRRL 32657 was determined by adding 2.5–20.0% (w/v) NaCl to the reaction mixture. Relative activities of the enzyme were calculated (Table 2).

Glutaminase activity increased by increasing the salt concentration to 2.5% and gradually decreased thereafter (Table 2).

Table 2
Effect of sodium chloride concentration on glutaminase activity.

NaCl (%)	Relative activity (%) ± SD
0.0	100 ± 2.50
2.5	130 ± 1.90
5.0	120 ± 3.30
10.0	109 ± 2.90
15.0	90 ± 1.76
20.0	60 ± 3.50

Salt concentrations of 2.5%, 5.0% and 10% resulted in higher activities as compared with reaction with no salt added. Addition of 15% and 20% NaCl decreased the activities to 90% and 60% of their original activities, respectively.

In the presence of 18% NaCl, glutaminases from *Escherichia coli*, *Pseudomonas fluorescens*, *Cryptococcus albidus*, *Aspergillus oryzae*, and *A. sojae* retained 65%, 75%, 65%, 20%, and 6% of their activities, respectively (Nandakumar et al., 2003). Weigand-Ziadé et al. (2003) found that salt enhanced glutaminase activity almost twofolds and 90% of the initial activity was remained at 15%. Sato et al. (2005) reported that glutaminase from *Bacillus subtilis* retained 90% and 80% from its original activity at 16% and 18% of the NaCl concentration, respectively. Wakayama et al. (2005) observed that *Stenotrophomonas maltophilia* strain NYW-81 L-glutaminase retained 90% of its activity in the presence of 16% NaCl. In the present study, the salt tolerance of the obtained enzyme was lower than that of the enzyme produced from *Micrococcus luteus* where 16% NaCl enhanced glutaminase activity 130% (Moriguchi et al., 1994).

Salt-tolerant enzymes may play potentially significant roles in industrial processes that require high salt environments like the soy sauce fermentation. Thus, salt tolerant L- glutaminases were patented for use in industrial processes (Sabu et al., 2000). Moriguchi et al. (1994) have proposed the use of salt tolerant L-glutaminase from bacteria as a possible alternative, since their enzymes could be halophilic rather than halotolerant allowing the use of high salt concentrations.

3.2.8. Effect of metal ions on glutaminase activity

Data (Fig. 6) reveal that Ca^{2+} and Na^{+} exhibited almost no effect on enzyme activity at the tested concentrations. Potassium showed a slight increase in activity of glutaminase especially, at the concentration of 0.5 mM. Zinc at the concentration of 0.1 mM did not affect the activity. However, at higher concentrations a gradual decrease in activity was noted and the relative activities were 98% and 94% for Zn^{2+} concentrations of 0.2 and 0.5 mM, respectively.

Ferric ions followed by ferrous showed the highest inhibition on glutaminase. The higher the concentration, the higher the

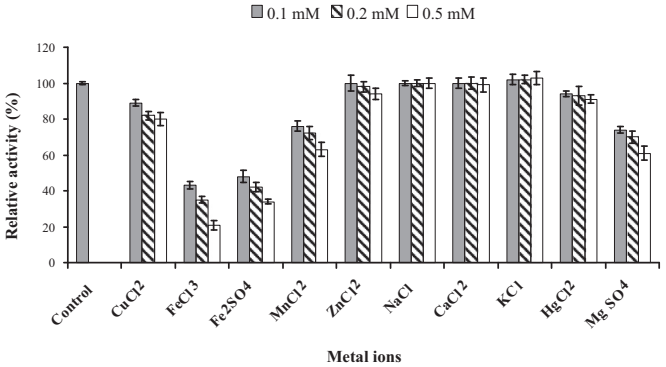

Fig. 6. Effect of various metal ions and their concentrations on glutaminase activity .

inhibition effect. In the presence of 0.1 mM ferric, a 57% inhibition was noted and by increasing concentration to 0.2 and 0.5 mM, the inhibition increased to 65% and 79%, respectively. Other metal ions (Cu^{2+}, Hg^{2+}, Mn^{2+} and Mg^{2+}) had moderate inhibition on activity and the percentage of inhibition was in the range of 9–39%. These results were in accordance with those of Wakayama et al. (2005) who worked on glutaminase of *Stenotrophomonas maltophilia* NYW-81. Kumar et al. (2012) recorded that glutaminase exhibited appreciable activity in the presence of K^{+}, Zn^{2+}, and Ni^{2+} and almost 80% of the activity was retained with all tested metal ions. Also, Yulianti et al. (2012) stated the positive effect of metal ions, e.g. Mn^{2+}, Mg^{2+}, and Co^{2+} (chloride salt) on the activity. Whereas, the addition of other metal ions e.g. Zn^{2+}, Fe^{3+} and Ca^{2+} decreased the activity.

4. Conclusion

The L-glutaminase from *Aspergillus oryzae* NRRL 32567 possesses good characteristics when compared to others previously reported in literature. It is active and stable over a wide range of pH and temperatures, highly salt-tolerant and L-glutamine acted as the best natural substrate for it. These properties render L-glutaminase from this source as a potential candidate as a flavor enhancer in food. This enzyme can be further used as enzyme supplementation therapy for cancer in pharmaceutical sectors.

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