Research Article

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COMPARATIVE CHARACTERIZATION OF L-ASPARAGINASE EXTRACTED FROM PLANT AND MICROBIAL SOURCES

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ABSTRACT

L-asparaginase has been used for the treatment of different leukemia such as Acute Lymphoblastic Leukemia. Unlike normal cells leukemic cells cannot synthesize asparagine on their own. Therefore, when L-asparaginase is injected into blood it breaks down asparagine to aspartic acid and ammonia and hence tumor cells die due to asparagine starvation. It is produced by both microbes as well as plants. Therefore, in the present study we compared the L-asparaginase extracted from plants as well as microbes. Among the plants *S. nigrum* showed the highest activity (52 IU/ml) whereas among microbes *B. subtilis* (MTCC-121) had the highest activity (43.11 IU/ml). Both the enzymes were kinetically characterized and it was found that plant enzyme was stable over pH and temperature variations. The effect of metal ions was same on both the enzymes whereas chelators enhanced the plant enzyme whereas had no effect on microbial enzyme. Plant enzyme showed the highest activity and was more stable to the environmental variations as compared to microbial enzyme.

Keywords: L-asparaginase, *S. nigrum*, *B. subtilis*, kinetic characterization.

INTRODUCTION

Hematopoietic malignancies such as Acute Lymphoblastic Leukemia (ALL) can be treated using L-asparaginase, an important chemotherapeutic agent. Acute Lymphoblastic Leukemia is a type of cancer in children arising due to expansion of lymphoid blasts and monoclonal proliferation in blood and bone marrow[1,2]. L-asparaginase synthetase is the only known enzyme for the synthesis of asparagine in cells. Normal cells are able to synthesize asparagine on their own but the cancer cells depend on the extracellular supply of asparagine from the blood. When L-asparaginase is injected into the blood it breaks down asparagine to aspartic acid and ammonia depriving the cancer cells of asparagine. Hence, the cancer cells die as a result of asparagine starvation[3,4]. It is available under brand names such as Elspar, Oncaspar, Erwinaze, Leunase, Medac, Crasnitin, Paronal, Kidrolase, Acrylaway, PreventASe, Cristanaspase and PEG-asparaginase[5,6]. It is a tetrameric protein produced and used by the organisms for the storage and transport of the nitrogen which is then utilized for the protein synthesis[7]. L-asparaginase is the first enzyme in humans to be studied for its antitumor properties. L-asparaginase isolated from *E. coli* and *Erwinia carotovora* is used for the clinical treatment of ALL but it suffers from a number of side-effects such as resistance, premature inactivation, allergic reaction, shorter drug duration and easy clearance from body[8,9]. Therefore, alternative sources such as plant enzyme are being searched for the isolation of L-asparaginase[10,2]. As compared to bacterial L-asparaginase plant enzyme has been not explored widely. Plant L-asparaginase is the major transport and storage compound for nitrogen and therefore helps in protein synthesis and nitrogen fixation[11]. Although the amino acid sequence of plant L-asparaginase is not similar to bacterial enzyme but it is 66% similar and 23% identical to human glycosylasparaginase[11]. The plant L-asparaginase is less toxic as compared to bacterial L-asparaginase[12].

MATERIALS AND METHODS

All the chemicals were purchased from Himedia and Merck, India. The microbial cultures were procured from IMTECH, Chandigarh.

Screening for L-asparaginase

Catharanthus roseus, *Withania somnifera*, *Pisum sativum* and *Solanum nigrum* were screened for L-asparaginase. The enzyme was extracted from different parts (roots, leaves, stem and fruits/flowers) of these plants using method given by Bano and Sivaramakrishnan[13]. The enzyme was collected by centrifugation at 12, 000 rpm for 12 min.

Bacillus subtilis (MTCC-121), *Erwinia caratovora* (MTCC-2760), *Enterobacter aerogenes* (MTCC-2822) and *Escherchia coli* K-12 (MTCC-1302) were procured from IMTECH, Chandigarh. The cultures were grown in M9 media and incubated at 37°C for 24 hrs. The cultures were centrifuged at 5, 000 rpm for 20 min. The crude enzyme was stored in PBS buffer (0.1 M, pH 7.2). The enzyme activity was calculated using nesselerization's method[14].

Kinetic Characterization of Enzymes

The effect of pH (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0), temperature (25, 30, 35, 40, 45, 50, 55, 60, 65 and 70°C) and asparagine concentration (10^{-10} to 10^{-1} M) was studied for both plant as well as bacterial enzymes.

Effect of Effectors on Enzyme Activity
Metal Ions

The effect of different metal ions (Ca^{2+}, K^{+}, Cu^{2+}, Na^{+}, Mn^{2+}, Mg^{2+}, Co^{2+} and Hg^{2+}) on L-asparaginase was studied by adding 10 mM of each metal ion into the reaction mixture and then calculating the residual activity (ratio of enzyme activity in presence and absence of effector). The control was taken in which no metal ion was added and its residual activity was considered as 100 %.

Chelators

The effect of different inhibitors [ethylenediaminetetraacetic acid (EDTA), urea, sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME) and Triton-X 100] on L-asparaginase was studied by adding 5 mM of each metal ion into the reaction mixture and then calculating the residual activity. The control was taken in which no metal ion was added and its residual activity was considered as 100 %.

RESULTS AND DISCUSSIONS

Screening for L-asparaginase

Different plants and their parts were screened for L-asparaginase activity and it was found that *S. nigrum* (Accession Number: K001152679) showed the highest activity i.e. 52 IU/ml (leaves) (Figure 1). It can be seen that leaves contain more amount of enzyme as compared to stem, roots and flowers/fruits. The similar results were found by Kataria *et al.*[15] in whose study *S. nigrum* showed the highest activity (58.62 U/mg).

B. subtilis (MTCC-121) showed the highest enzyme activity (43.11 IU/ml) whereas *E. aerogenes* (MTCC-2822) had the least enzyme activity (21.45 IU/ml) after 24 hrs incubation. With the increased incubation time the enzyme activity reduces (Figure 2). The enzyme activity was higher than reported by Jia *et al.*[16] which was 9.98 U/ml.

Kinetic Characterization of Enzymes
Effect of pH

It was found that optimum pH for *S. nigrum* L-asparaginase was 7.5 whereas for *B. subtilis* (MTCC-121) it was pH 7.0 (Figure 3). The optimum pH for L-asparaginase extracted from *Phaseolus vulgaris* was 8.0[17]. It is also clear from figure 1 that plant enzyme is more stable over pH range whereas the enzyme activity of microbial enzyme gradually decreases with the increasing pH. The results were similar to the fact that microbial enzyme shows highest activity at pH 8.0-10.0[18,19]. The optimum pH for *B. subtilis* (MTCC-2822) was 7.5 and 86 % activity was retained at pH 8.0[16].

Effect of Temperature

The optimum temperature for plant as well as microbial L-asparaginase is 40°C (Figure 2). Similarly, L-asparaginase extracted from *B. subtilis* (MTCC-2822) showed the highest activity at 40°C[16]. Moreover, it is evident from the figure 4 that plant enzyme is more thermally stable as compared to the microbial enzyme. The results were similar to *Vigna unguiculata* which had optimum temperature at 40°C[20]. L-asparaginase extracted from *P. vulgaris* had the optimum temperature at 37°C[17]. *Pectobacterium carotovorum* and *Pseudomonase aeruginosa* were used for the production of L-asparaginase which also showed the highest activity at 40°C[21,19].

Effect of Substrate Concentration

The K_m and V_{max} value for L-asparaginase extracted from *S. nigrum* was 6.62 mM and 52.63 µM/min respectively (Figure 5a) whereas L-asparaginase extracted from *B. subtilis* (MTCC-121) had K_m 6.17 mM and V_{max} 45.45 µM/min (Figure 5b). K_m and V_{max} for L-asparaginase extracted from *P. vulgaris* were 6.72 mM and 0.16 µM/ml[17]. The K_m values for *Erwinia carotovora* and *E. coli* L-asparaginase were 7.14 and 3.5 mM respectively[22,23]. L-asparaginase extracted from *B. subtilis* (MTCC-2822) had K_m 0.43 mM and V_{max} 77.51 µM/min[16].

Effect of Effectors on Enzyme Activity
Metal Ions

Effect of different metal ions on L-asparaginase extracted from *S. nigrum* and *B. subtilis* (MTCC-121) was studied and it was found that metal ions have similar effects on both enzymes (Figure 6). Ca^{2+}, K^{+}, Na^{+} and Co^{2+} ions enhanced the enzyme activity with K^{+} ions giving 60 % residual activity for plant L-asparaginase and 40 % for microbial L-asparaginase. Cu^{2+}, Mg^{2+}, Mn^{2+} and Hg^{2+} showed the inhibitory effect on L-asparaginase activity. Cu^{2+} ions slightly reduced the enzyme activity whereas Hg^{2+} ions inhibited the enzyme activity completely. L-asparaginase extracted from *V. unguiculata* was inhibited by Mn^{2+}, Hg^{2+} and Ba^{2+} ions whereas activated by Co^{2+} and Ni^{2+} ions[20]. Similar results were found in the study conducted by Jia *et al.*[16] where Fe^{3+} ions were the strongest inhibitors. K^{+} enhanced the L-asparaginase activity of *P. vulgaris* with 150 % residual activity whereas Hg^{2+} inhibited the enzyme[17]. Co^{2+} ions enhanced the enzyme activity by 27 % in *Pseudomonas pseudoalcaligenes*[24].

Chelators

Different chelators were incubated in the reaction mixture and it was found that EDTA enhanced the activity of L-asparaginase extracted from *S. nigrum* but had no effect on microbial L-asparaginase (Figure 7). Similarly, β-ME slightly increased activity of plant L-asparaginase but slightly inhibited microbial enzyme. Urea, SDS and Triton-X 100 inhibited both the enzymes with urea as the strongest inhibitor. The results were similar to the study in which EDTA had no effect on L-asparaginase extracted from *B. subtilis* (MTCC-2822)[16]. EDTA partially inhibited L-asparaginase from *P. vulgaris*[17] whereas had no effect on L-asparaginase from *P. carotovorum*[21]. EDTA inhibited L-asparaginase activity in *P. pseudoalcaligenes*[24].

CONCLUSION

Out of the different plants screened for L-asparaginase *S. nigrum* had the highest enzyme activity whereas among the microbial sources *B. subtilis* (MTCC-121) showed the highest enzyme activity. The enzymes were kinetically characterized and found that plant enzyme was more stable over pH as well as temperature variations as compared to the microbial enzyme. K^{+}, Na^{+}, Ca^{2+} and Co^{2+} ions enhanced the enzyme activity whereas Cu^{2+}, Mg^{2+}, Mn^{2+} and Hg^{2+} inhibited the L-asparaginase. EDTA and β-ME acted as the enhancers whereas urea, SDS and Triton-X 100 were the inhibitors. Overall, it can be said that in comparison to the microbial enzyme the plant enzyme is much more stable over different parameters.

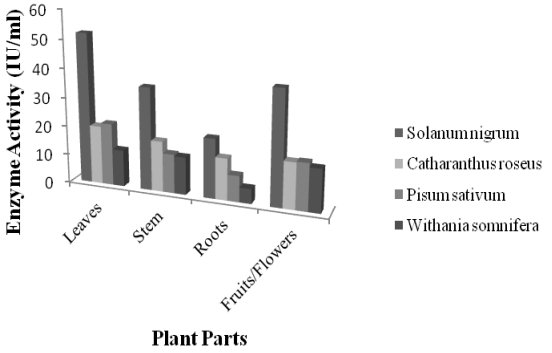


Figure 1: Enzyme Activity of Different Plant Sources

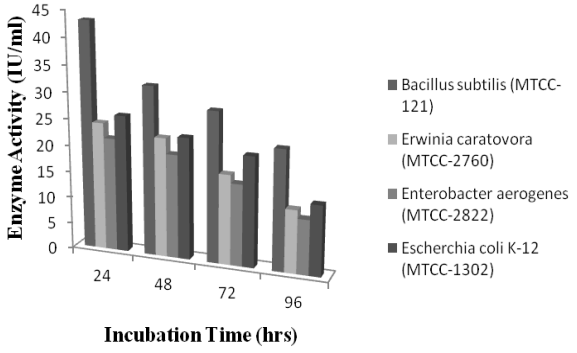


Figure 2: Enzyme Activity of Different Microbial Sources

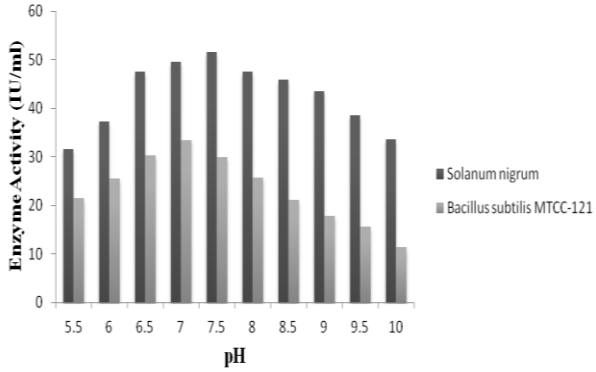


Figure 3: Effect of pH values on Activity of L-asparaginase Extracted from *S. nigrum* and *B. subtilis* MTCC-121

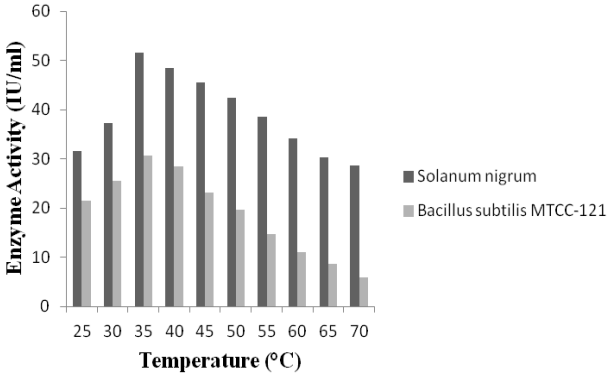
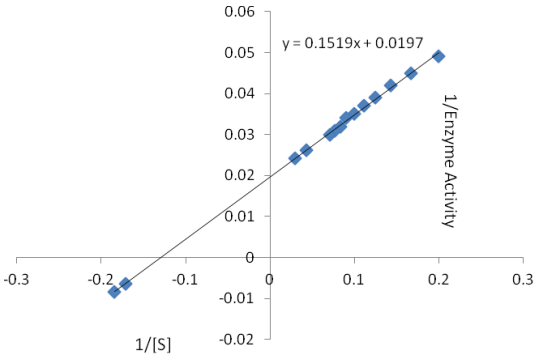


Figure 4: Effect of Temperature on Activity of L-asparaginase Extracted from *S. nigrum* and *B. subtilis* MTCC-121



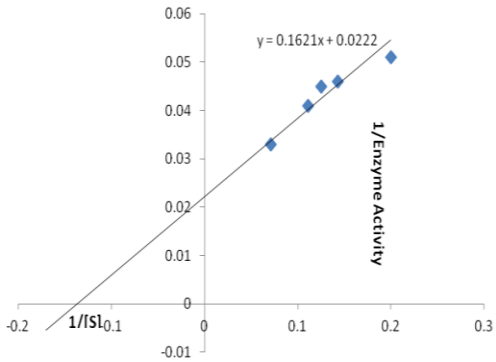


Figure 5: Lineweaver-Burk Plots (a) *S. nigrum* (b) *B. subtilis* MTCC-121

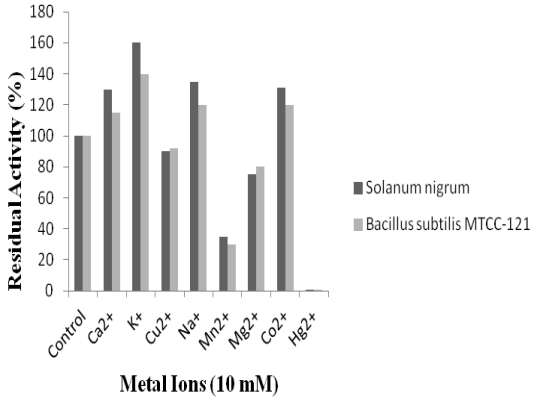


Figure 6: Effect of Metal Ions on Activity of L-asparaginase Extracted from *S. nigrum* and *B. subtilis* MTCC-121

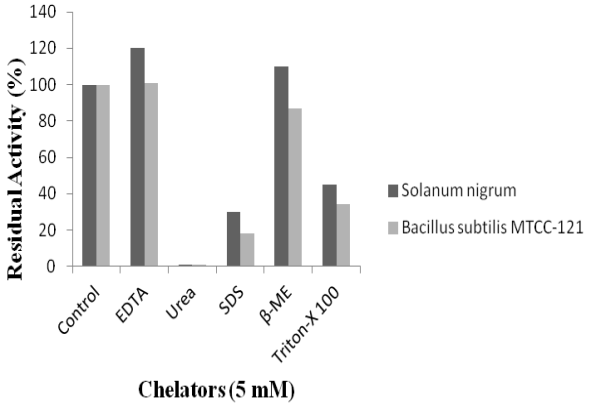


Figure 7: Effect of Metal Ions on Activity of L-asparaginase Extracted from *S. nigrum* and *B. subtilis* MTCC-121

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