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Original Research Article

Production and Purification of Anticancer Enzyme L-Asparaginase from *Bacillus firmus* AVP 18 of Mangrove Sample through Submerged Fermentation

Pallavi Rudrapati and Amrutha V. Audipudi*

Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, A.P., India
**Corresponding author email id: audipudi_amrita@yahoo.com*

Keywords

Bacillus firmus, Submerged Fermentation, Optimization and Shake culture fermentation, Purification

A B S T R A C T

L-asparaginase is used in the treatment of cancer, especially for acute lymphoblastic leukemia. Bacterial species were isolated from Nizampatnam mangrove sample and screened for its ability to produce the extracellular L-asparaginase enzyme. Effect of pH and temperature were investigated in the production of enzyme. Highest yield of enzyme was observed when the pH of the fermentation cycle raised above 9 at 37°C. Effective production was observed while using Dextrose and Peptone as carbon and nitrogen source.

Introduction

In recent years enzymes have gained great importance in clinical research. L-asparaginase is one of them which are widely present in nature. L-asparaginase (EC3.5.1.1) catalyzes the hydrolysis of L-asparagine into aspartic acid and ammonia. L-asparaginase has been a clinically satisfactory antitumor agent for the valuable treatment of acute Lymphoblastic leukemia (ALL) and lymph sarcoma (Pieters, *et al.*, 2011). L-asparagine is an essential amino acid for the production of protein in tumor cells whereas the growth of normal cell is independent of its requirement. L-asparaginase can be produced within the cells by an enzyme called asparagine synthase are can be absorbed from the outside. Lymphatic tumor cell required huge amount of asparagine to keep up their rapid malignant growth. In the presence of L-asparaginase tumor cell get deprivated and cannot survive (Ali *et al.*, 1994, Berenbaum, *et al.*, 1970 and Box *et al.*, 1978).This fact suggests that Lasparaginase enzyme used as anti tumor or anti leukamatic drug.

Bacterial L-asparaginases are enzymes of high potency used in treating various kinds of cancers, mainly acute lymphoblastic leukemia. Bacterial L-asparaginases are either high affinity periplasmic enzyme or low affinity cytoplasmic enzyme.

A high affinity periplasmic L-asparaginase is particularly effective in certain kinds of

cancer therapies (Maria *et al.,* 2004 and stecher *et al.,* 1999).

L-asparaginase is widely distributed among the microorganism, animals, and plant. The microorganisms are a better source of L-asparaginase because they can be cultured easily (Howard *et al.,* 1968, Peterson *et al.,* 1996, Tosa *et al.,* 1971, Davidson L *et al.,* 1977, Dejong *et al.,* 1972 and Dhevendaram *et al.,* 1999).*Erwinia caratovira*, *Corynebacterium glutamicum*, *Bacillus* sp, *Psudomonas stutzeri*, and *E. coli* are most commonly used microorganisms for the production of L-asparaginase (Shwu *et al.,* 1998 and Appel, *et al.,* 2007). L-asparaginase from *E. chrysanthemi* is pharmacologically active and that from *E. coli* is also having anti tumor effect.

Since these two L-asparaginases possess different immunological specification and the availability to provide an important alternative therapy. Unlike other chemotherapy agents, it can be given as intramuscular, intravenous or subcutaneous injections without fear of any side effect or tissue irritation (Hill *et al.,* 2002).

The exact mechanism of L-asparaginase is still unknown although hydrolysis proceeds in two steps via beta-acylenzyme intermediates (Kornbrust, 2009). L-asparaginase also plays important role in biosynthesis of aspartic acid family of amino acids. Different types of L-asparaginase can be used for different pharmacological and industrial application. L asparaginase is used to reduce the formation of acrylamide (El-Bessoumy, 2014). The main side effect is hypersensitivity or allergic reactions; anaphylaxis is a possibility. Additionally it can also be associated with a coagulopathy as it decrees protein synthesis, including synthesis of anti coagulant factor, leading to bleeding or thrombolytic events such as stroke (Pradeep, *et al.,* 2010 and Heesgen *et al.,* 1996).

Mechanism of Action

In normal cells, the asparaginase used for protein synthesis is generated from aspartate by asparagine synthase. Outside the cell asparagines is converted into aspertate by Lasparaginase. L- asparaginase causes selective toxicity for tumor cell because they lack L- asparaginase synthase (Abuchowski *et al.,* 1984 and Alegre *et al.,* 1993). L-asparaginase catalyses the hydrolysis reaction to convert L-asparaginase into L aspertate and ammonia (Boos *et al.,* 1996). (Fig. 1). Asparagine is required for cell survival and DNA synthesis; however, most of the cells are capable to synthesizing asparaginase from glutamine (Roberts *et al.,* 1968 and Giovanni, *et al.,* 1973).

Acute lymphoblastic leukemia cells lack adequate level of the asparagines synthase and cannot survive in asparagine depletion. Asparginase is cycle specific for the G1 of cell cycle (Illarionova, *et al.,* 1980).

Properties

L-asparaginase catalyses the deamination reaction to produce L- aspartic acid and ammonia. L-asparaginases are mainly tetrameric in nature. In some harsh condition like high PH and freeze drying changed the tetramer structure of the enzyme in to monomer (Hellman, *et al.,* 1983, Hess J *et al.,* 2002, Hill J *et al.,* 2002, Howard *et al.,* 1968, Joner, *et al.,* 1976 and Kamble, *et al.,* 2012). For enzyme activity ionization and deionization of the functional group of the active center are responsible. L-asparginase has anticancer and antitumor property. It is used as anticancer agent because it is biodegradable and non-toxic (Jones *et al.,* 1977).

Materials and Methods

Materials

M9 medium was obtained from Himedia laboratories, Mumbai. Standard L-asparaginase, L-Asparagine monohydrate and Nesslers reagent was purchased from National scientific products, Guntur. Di-Potassium hydrogen phosphate, sodium potassium tartrate and remaining chemicals and reagents were obtained from Qualigens.

Sample collection

Mangrove soil samples were aseptically collected from mangrove sediments of Nizampatnam, Guntur, A.P, India. In a sterile container for the isolation of L-Asparaginase producing organisms under laboratory conditions.

Isolation of Bacteria

Isolation of bacteria were performed by the serial dilution technique Aneja,K.R. *et al.*,2003.using nutrient agar medium (Peptone, 5.0 g; Beef-extract, 3.0 g; Sodium chloride, 5.0 g; agar-agar, 20.0 g per liter of distilled water). The sterilization of the media components were carried out in an autoclave at 121°C at 15 lbs. pressure for 30 minutes. Approximately 20 ml of media was poured into pre - sterilized Petri dishes and allowed to solidify. The sample (1g) was serially diluted up to 10-6 dilutions. A 0.1 ml of this dilution was aseptically spread over the surface of poured petridishes having nutrient agar medium. The plates were then incubated at 37°**C** for 48 hours. A colony appeared with characteristics of bacterial morphology was isolated and purified using nutrient agar medium. After purification, all the isolated bacteria were maintained on nutrient agar slants and stored at 4°C for further use.

Screening of L-asparaginase producer by plate assay

The strains obtained from the above steps were subjected for rapid screening of L-asparaginase production by plate assay method. The modified M9 medium was supplemented with phenol red dye (2.5% prepared in ethanol and the pH was adjusted to 7.0). The media was autoclaved and plates were prepared. Control plate was maintained without asparagine. The plates were inoculated with bacterial strain isolated from the mangrove sample. The zone was observed after 48 hrs (Gulati R *et al.*, 1997).

Molecular Identification

Pure culture of AVP 18 bacterial isolate was grown until log phase achieved and genomic DNA was isolated essentially (Bazzicalupo *et al.,* 1995). The amplification of 16S rRNA gene was done by using universal bacterial primer 1492R (5´-TACGGYTACCTTGTTACGACTT-3´) and 27F (5´ AGAGTTTGATCMTGGCTC AG-3´) as per the conditions (Pandey P *et al.*, 2005). The PCR product was sequenced at Macrogen South Korea. The sequences obtained were compared with those from the GenBank using the BLAST program and Phylogenetic trees reconstructions were obtained by the Neighbor joining method 1000 bootstrap replicates were performed to assess the statistical support for each branch in the tree (Astchul 1990,Tamura 2007).

Preparation of Production Medium

The medium contained Na2HPO4.2H2O, 6.0 g; KH2PO4, 3.0 g; NaCl, 0.5 g; L-asparagine, 10.0 g; 1moll-1 MgSO4.7H2O, 2.0 ml; 0.1 M solution of CaCl2.2H2O, 1.0 ml; 20% glucose stock, 10.0 ml; agar 20.0 g. per liter of distilled water. The medium was supplemented with 0.005% phenol red dye

(prepared in ethanol) and the pH was adjusted to 6.2 using 1N HCl. 9 ml of medium was poured in each tube and were sterilized. The tubes were inoculated with test organisms by using one loopful culture of bacteria and incubated at 37°C for 5 days. A set of tubes were also run as control without L-asparagine. The modified - M9 medium contains 1% L-asparagine as sole source of nitrogen. L-asparaginases hydrolyses L-asparagine into L-aspartic acid and ammonia.

Production of L-asparaginase by Submerged Fermentation

The Erlenmeyer flask (250ml) having 50 ml broth were inoculated with spore suspension after autoclaving and incubated at 150 rpm for 72 hours at 37°C. Uninoculated medium was served as control. At the end of incubation, culture filtrates were obtained by centrifugation at 8000 rpm for 15 min.

The supernatant was then used as crude extract for L-asparaginase activity and stored at 4°C for further use. The production of L-Asparaginase was studied at different inducers and enhancers like pH ranges, temperature, Salinity concentrations, Carbon sources, Amino acids, Nitrogen sources, Phosphate sources, Metal /Mineral salts

Determination of L-asparaginase Activity

The rate of hydrolysis of L-Asparagine was determined by measuring the ammonia released using Nessler's reaction (Mashburn *et al.,* 1964). The color reaction was allowed to develop for 10 min and the absorbance read at 480 nm with a spectrophotometer. The ammonia liberated was extrapolated from a curve derived with ammonium sulphate. One unit (U) of L-Asparaginase was defined as that amount of enzyme which liberates 1 µ mole of ammonia per minute under the assay conditions (Theantana *et al.,* 2007).

Partial Purification of Enzyme

The culture filtrate was filtered through Whatman No. 1 filter paper and centrifuged at 8000 rpm for 10 min at 4°C. The culture filtrate (crude enzyme) was brought to 45 per cent saturation with ammonium sulphate at pH 8.4 and kept overnight in a cold room at 4°C. It was thereafter subjected to centrifugation at 8000 rpm for 10 min at 4°C. The precipitate was discarded, while the supernatant was brought to 80 per cent saturation with ammonium sulphate and centrifuged at 8000 rpm at 4°C for 10 min. The precipitate from this step was collected and stored at 4°C.

Kinetics of the Partial purified L-asparaginase Enzyme

To measure the kinetics of L-asparaginase, Michaelis constant (Km) and Maximal velocity (Vmax) of the partial purified enzyme was determined. They are one of the important parameters for the evaluation of the potential usefulness of the enzyme for anti-leukemic therapy. They were determined using L-asparaginase as substrate in the range of 0.01M-1M concentration. Each reported velocity I the mean of at least three measurements. The apparent Km was determined (Basha *et al.,* 2009).

Bio processing of L-Asparaginase under Solid State Fermentation (SSF)

The culturable microorganisms can be manipulated and processed due to their small size and huge reproduction capabilities (Kelecom *et al.,* 2002). The scaling up and mass production are relatively easy in microorganisms where they can be grown in large-volume. Many microorganisms can be stored for an

indefinite time, ensuring availability of the targeted source organism. The microorganisms can be manipulated both physico-chemically and genetically to increase yields of desired natural products (Duval *et al.,* 2002).Coconut oil cake, Coffee, Green tea, Groundnut oil cake, Red gram and Sesame oil cake, procured from a local oil extracting unit of Guntur, Bapatla and Narasaraopet, Andhra Pradesh, India were used as the substrate. The substrate was dried at 60°C for 72h to reduce the moisture content to approx. 5% and ground to a desired size. Ten grams of each substrate was measured into 250 ml Erlenmeyer flasks into which a supplemental salt solution was added properly to get the desired moisture level. The salt solution composed of 6.0 g/L Na2HPO4.2H2O, 3.0 g/L KH2PO4, 0.5 g/L MgSO4.7H2O, and 0.5 g/L NaCl. L-asparagine was supplemented as inducer for synthesis of enzyme L-asparaginase (Kharwar R.N *et al.,* 2011).Content was mixed properly and autoclaved at temperature 121°C (pressure 15 psi) for 15 min. The sterilized fermentation media was inoculated with 2.0 ml of Inoculum, mixed thoroughly and incubated at 37°C for 5 days in a stationary condition. Each experiment was done in triplicate.

Extraction of Crude Enzyme

The recovery of crude L-asparaginase from the fermented material was done by simple extraction method. For this, the fermented substrate was mixed thoroughly with 50 ml of 50 mM phosphate buffer (pH 7.0) and the contents were agitated for 1 h at room temperature in a rotary shaker at 150 rpm. The liquid was filtered off through Whatmann No.1 filter paper and the resulting clear filtrate. The filtrate was then centrifuged at 8000rpm for 15 min to get enzyme as clear supernatant (Moorthy V. *et al.,* 2010).

Assay of L-asparaginase Activity

Enzyme activity of the bacterial isolate AVP 18 was determined by quantifying ammonia production with Nessler's reagent. Add 0.1ml sample of culture filtrate (enzyme solution), to 0.9 ml of 0.1 M Tris-hydroxy methyl amino methane buffer (pH 8.5), and 1 ml of 0.04 M L-asparagine and incubated for 10 min at 37°C. The reaction is stopped by the addition of 0.5 ml of 15% (w/v) Trichloroacetic acid. After centrifugation, a 1 ml portion of the supernatant fluid is diluted to 3 ml with distilled water and treated with 1.0 ml of Nessler's reagent and 1.0 ml of 2.0 M NaOH. The absorbance of the resultant color was read after 20 min at 480nm in UV/Visible Spectrophotometer. The OD was then compared to a standard curve prepared from solutions of ammonium sulfate as the source. Blank was prepared by without asparaginase enzyme sample (Produced in production medium). One unit (IU) of L-asparaginase is that amount of enzyme which liberates 1 µmole of ammonia in 1 min at 37°C.

Results and Discussion

Isolation of Bacteria

Bacterial strains were isolated from soil samples collected from 10 ft depth of Nizampatnam marine soil sediment of Guntur district. The soil samples were serially diluted plated.

Screening of L-asparaginase producer by plate assay

Isolates obtained were screened for the production of the enzyme L-asparaginase by using plate assay method (qualitative method). Results obtained showed higher intensity of pink coloration for nine out of forty seven cultures tested (Figure 1).

Cultures giving positive test with plate assay method were then checked for enzyme activity quantitatively. Results obtained showed that one of the nine tested cultures gave higher enzyme activity and therefore it was selected for further optimization studies on L-asparaginase production.

Molecular Identification

The bacterial strain AVP 18 was classified to be *Bacillus sp.* A 1466 bp PCR product of gene was amplified from the genomic DNA of AVP 18.A sequence similarity showed that the 16srDNA gene sequence of AVP 18 had 99% similarity to the 16srDNA of *Bacillus firmus* strain and *Bacillus* species PPB2(AC:HM771657).The sequence was blast in NCBI and for analysis. Based on phylogenetic analysis revealed that AVP 18 was closely related to *Bacillus firmus* (Figure 2) and sequence was deposited in NCBI as *Bacillus firmus* AVP 18 with accession number KF527832.

The selected AVP18 bacterial strain with anticancer properties was found to be with L- asparaginase activity, an essential tumor controlling enzyme. Our results supported the hypothesis that the marine bacterial strains with medicinal properties.

Determination of L-Asparaginase activity

The L-Asparaginase activity was assayed by Nesslerization method, a most common method for activity estimation. This was performed by quantifying ammonia formation in a spectrophotometric analysis at 450 nm for the respective concentrations and the observed values are shown in optimisation studies.

Enzyme Kinetics of Partial purified L-Asparaginase Enzyme

An attempt was made to evaluate the extent of improvement in production of L-asparaginase in the modified formulated production media with necessary inducers and enhancers. 5 folds of enhancement in L-asparaginase production observed with modified production medium indicating highly significant improvement so far observed. Enzyme kinetics, Vmax and Km values were studied at different substrate concentrations (0.01-1 M) and at different incubation periods (24 hrs, 48 hrs and 72 hrs). At 24 hrs of incubation, Vmax value of L-asparaginase is 108.08 and Km value is 0.09. At 48 hrs, Vmax is 170.95 and Km value is 0.1 and at 72 hrs AVP 18 showed 201.83 Vmax value and 0.3 Km value. Enzyme kinetics study revealed that L-Asparaginase of AVP 18 showed Vmax at 0.3 M substrate (Figure 3). Enzymatic activity of the strain

AVP18 measured at physiological temperature showed lower Km values. The Km values obtained were closer those of some mesophilic L-asparaginase of earlier studies.

Bio processing of L-Asparaginase under Solid State Fermentation (SSF)

Solid state fermentation (SSF) has gained fresh and plentiful attention of researchers to overcome the drawbacks of submerged fermentation. SSF has several advantages over submerged fermentation such as lesser energy requirements, very low risk of bacterial contamination, lower need of water and less environmental concerns regarding the disposal of solid waste Doelle *et al.*, 1992. Additionally, the utilization of agro-waste solid as a substrate for carbon and energy requirement under SSF makes this approach environmental friendly. Because of optimum moisture level and low volume of medium per unit weight agro industrial byproducts are generally used as solid

substrates for bioprocessing of enzymes. SSF of Lasparaginase of fungal origin was extensively studied than bacterial L-asparaginase.

Earlier studies revealed that incubation period for production of L-asparaginase production varies from species to species *Aspergillus terrus* showed maximum L-asparaginase production at 96 hrs of incubation under SSF and showed variation in production of L-asparaginase with substrate and incubation period Balasubramanian, *et al.,* 2012, Akilandeswari, 2012 *et al.*, Siddalingeshwara, *et al.,* 2011 and Khamna, *et al.,* 2009 reported asparaginase production by isolated *Bacillus circulans* MTCC 8752 under solid state fermentation using different agricultural materials like red gram husk, Bengal gram husk, coconut, and groundnut cake Hymavathi, *et al.,* 2009.

In present study an attempt was made for maximum production of L-asparaginase with 6 different solid substrates of different composition of carbon and energy sources.

Out of the six substrates tested for their suitability to support SSF production of L-asparaginase, Ground nut oil cake appeared to be the best substrate supporting maximum enzyme activity of 185.29 IU after 5 days of incubation. Green tea supported 173 IU, Sesame oil cake supported 146.32 IU, Coffee supported 139.7 IU, Red gram supported 131.25 IU, while Coconut oil cake supported 124.26 IU for L-asparaginase activity (Figure 4). As maximum activity was seen using Red gram husk therefore, Red gram husk might be the potential solid substrate for bioprocessing.

Production of L-asparaginase by Submerged Fermentation

Production of L-asparaginase was found to be maximum at 37°c (24.26 IU/ml) and varies with incubation period at 37°c indicating optimization of incubating period also exhibit a significant role (Figure 5). The significance of the incubation temperature in the development of sub merged fermentation process is such that it could determine the effects of inhibition, cell viability and death. However, the enzyme production reduced gradually with further increase in incubation temperature. This may be due to heat that accumulates in the medium, because of poor heat dissipation which could lead to a further drop in the oxygen level and thereby reducing the growth of the test organism. The optimum temperature and stability of enzyme to temperature was determined by gaffer protocol. The optimum temperature for L-asparaginase activity is 37°C L-asparaginase active at a wide range of temperature condition from 30 to 75°C Maladkar, 1993 and Gallogher, 1989. Beyond this temperature the enzyme becomes unstable. This property of enzyme plays important role for complete elimination of asparagine from the patient body when they treated with L- asparaginase in vivo. The residual activity is 100% at 70°C for 30 and 60 minutes At 77°C it retain 100% activity Frank, B. H., *et al.*,1977 and Sarqius *et al.,* 2004 have reported 30°C is suitable for L-asparaginase production through submerged fermentation by using *A. terreus* and *A. tamari*. Siddalingeshwara (2010) reported optimized temperature as 30°C by *Emericella nidulans*. Yogendrasingh *et al.,* (2012) observed the maximum activity at 30ºC by *Bacillus aryabhattai* strain ITBHU02

Enzyme production of AVP 18 showed variation at different pH. L-asparaginase production was found to be maximum (29.04 IU/ml) at pH 9 and observed to be gradually decreased beyond pH 12(Figure 6) Growth and metabolism along with enzyme

production is governed by an important factor called pH. Different pH optima and any modification in their pH optima could result in a decrease in their enzyme activity. Experiments were carried out to find the optimum pH in order to maintain the favourable conditions for increased L-asparaginase production. This was established by carrying out the fermentation by varying the pH from 5-14. The L-asparaginase activity below pH 8 would not be expected to be effective for the treatment of the tumor patient. The membrane bound L- asparaginase from *T. pyriformis* acts optimally at pH9.6. The enzyme activity is slightly lowered at pH value of 7.5 to 8.0. *E. carotovora* L- asparaginase is evidently more stable than *E. coli* enzyme in the alkaline pH region. G.Thirumurugan *et al.*, 2011 reported an optimum asparaginase production at pH 8.0 by *Aspergillus terreus*. Selvakumar 2011 observed peak activity of asparaginase at pH 8.0 by *Streptomyces noursei* MTCC 10469.

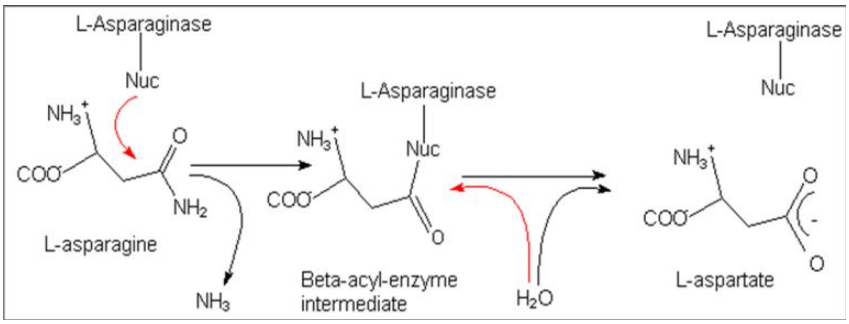


Fig.1 Screening of L-asparaginase producer by plate assay

Control	L-Asparaginase Producer
	

Fig.2 Molecular Identification Phylogenetic tree of AVP 18

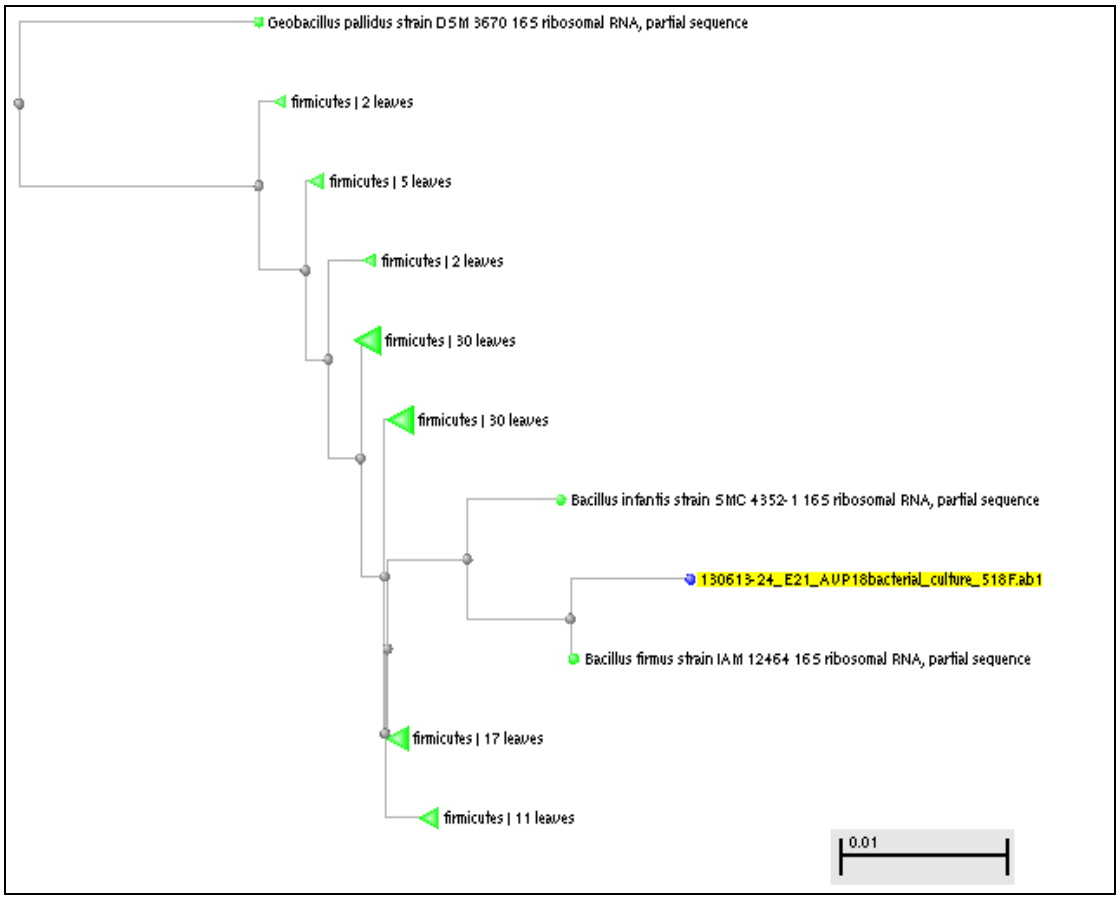


Fig.3 Enzyme Kinetics of Partial purified L-Asparaginase Enzyme

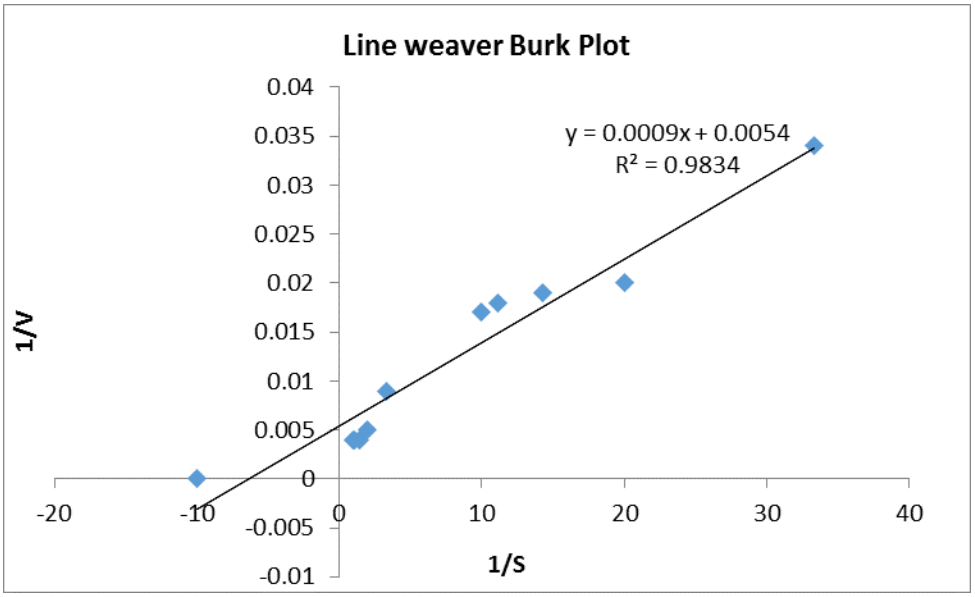


Fig.4 Bio processing of L-Asparaginase under Solid State Fermentation (SSF)

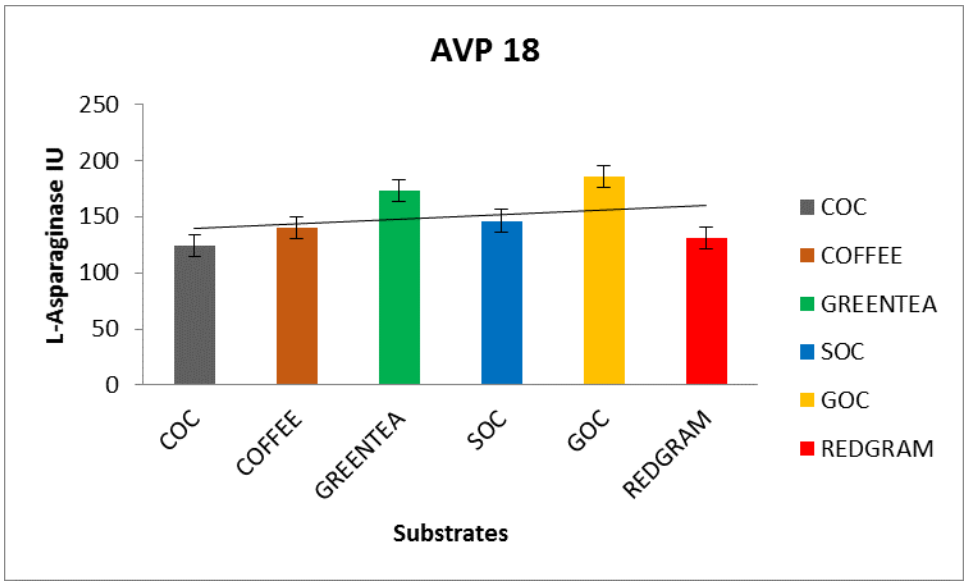


Fig.5 Production of L-asparaginase by Submerged Fermentation Effect of Temperature

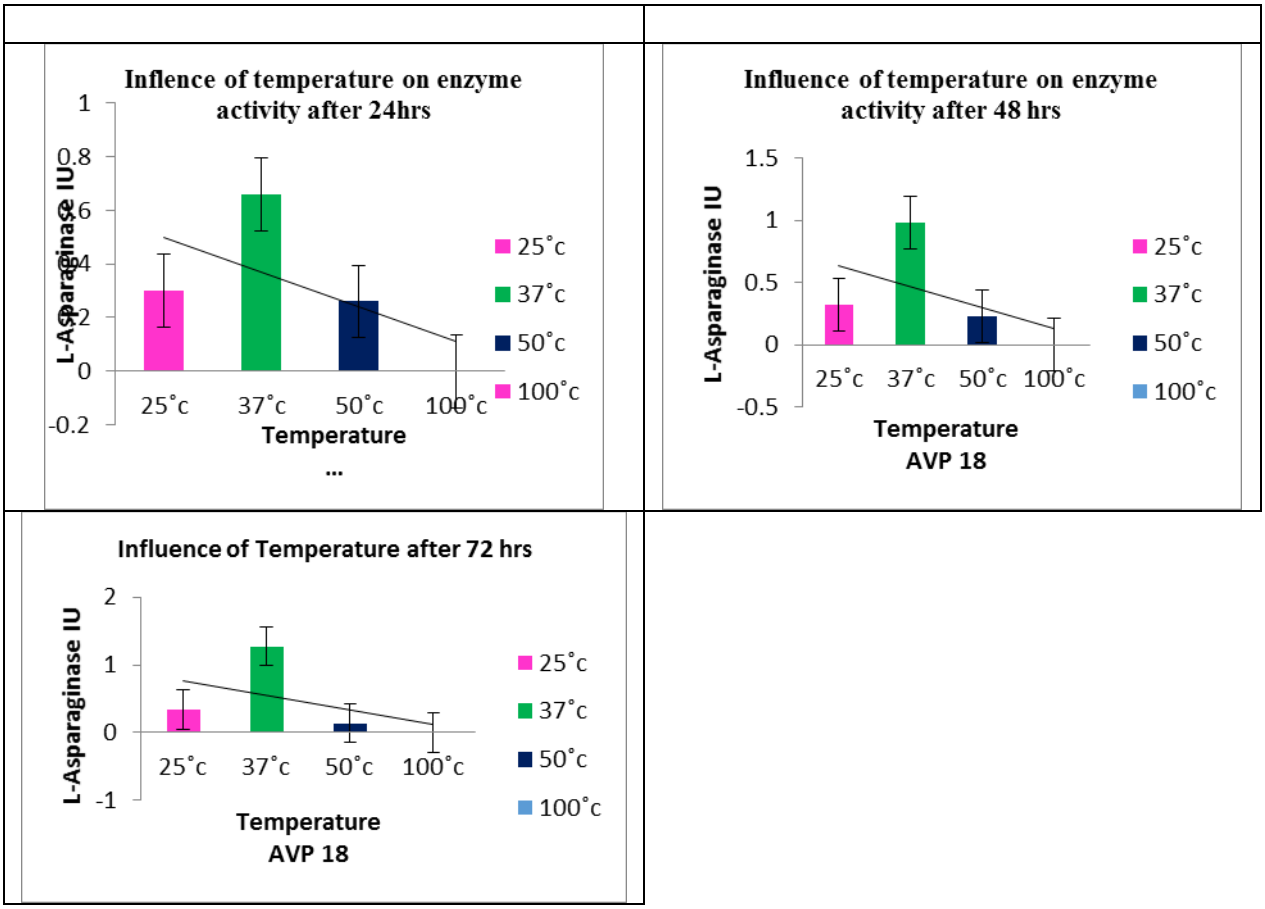


Fig.6 Effect of pH

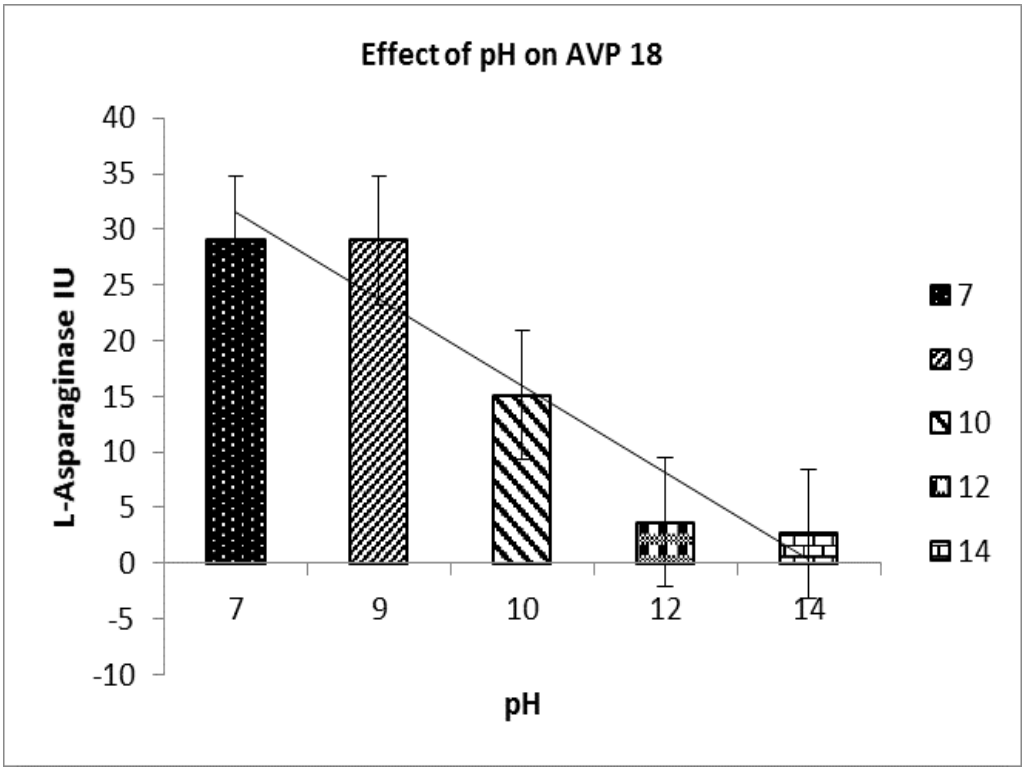


Fig.7 Effect of Salinity Concentrations

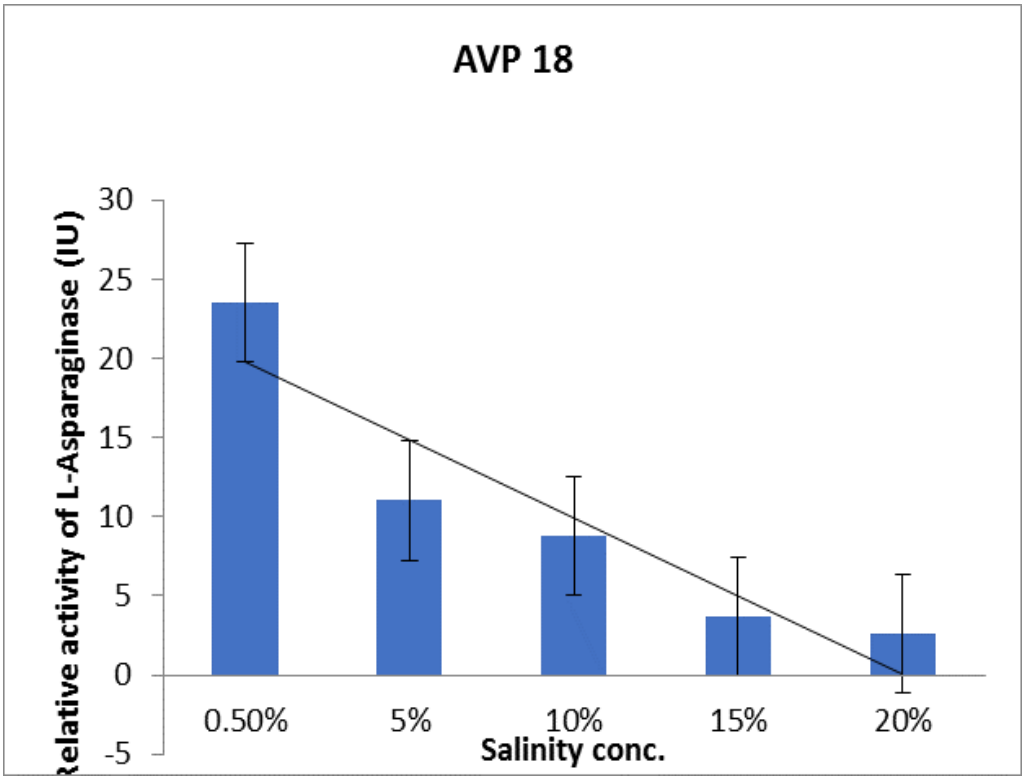


Fig.8 Effect of Carbon sources

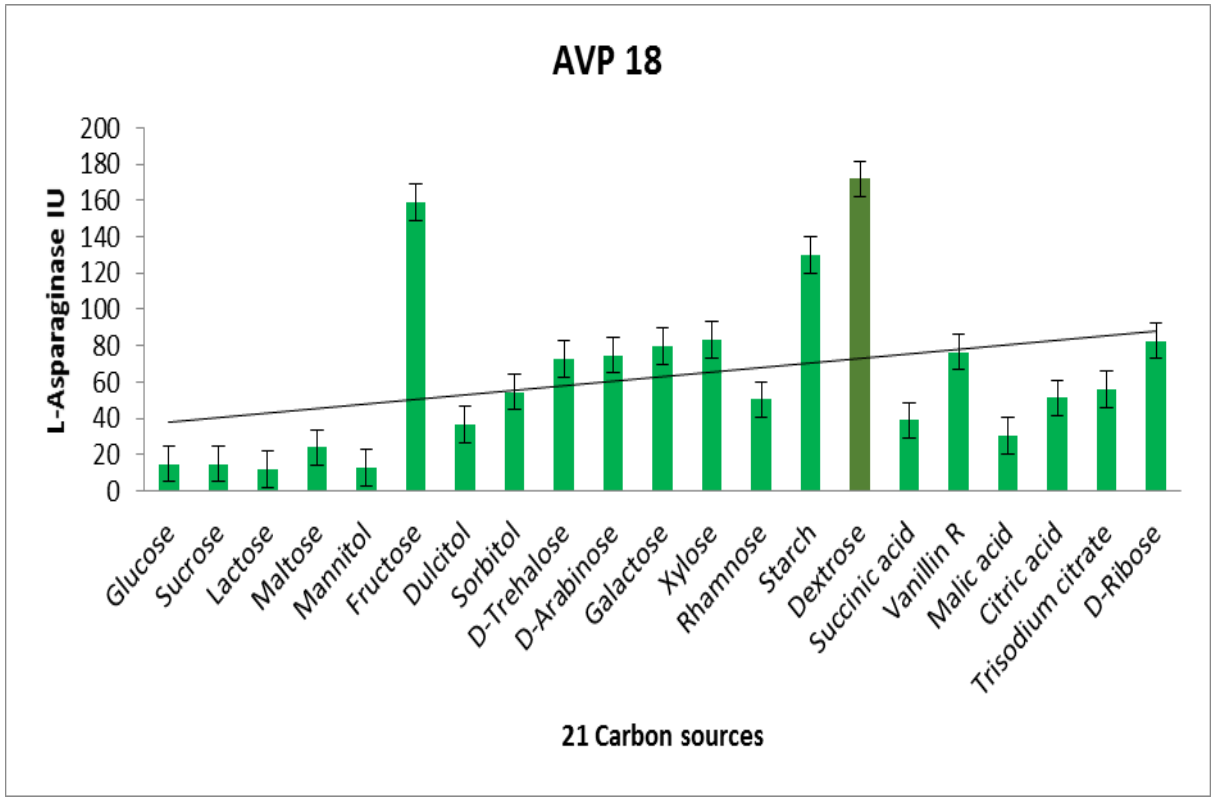


Fig.9 Effect of Nitrogen sources

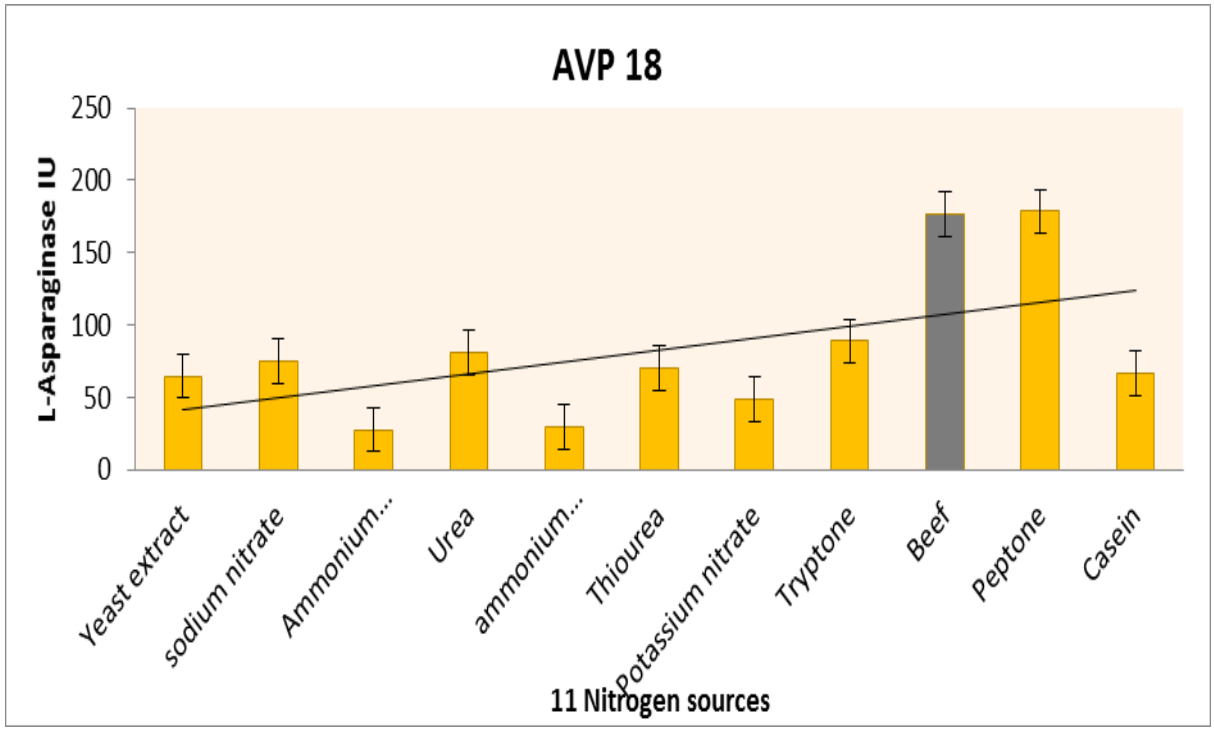


Fig.10 Effect of Amino acids

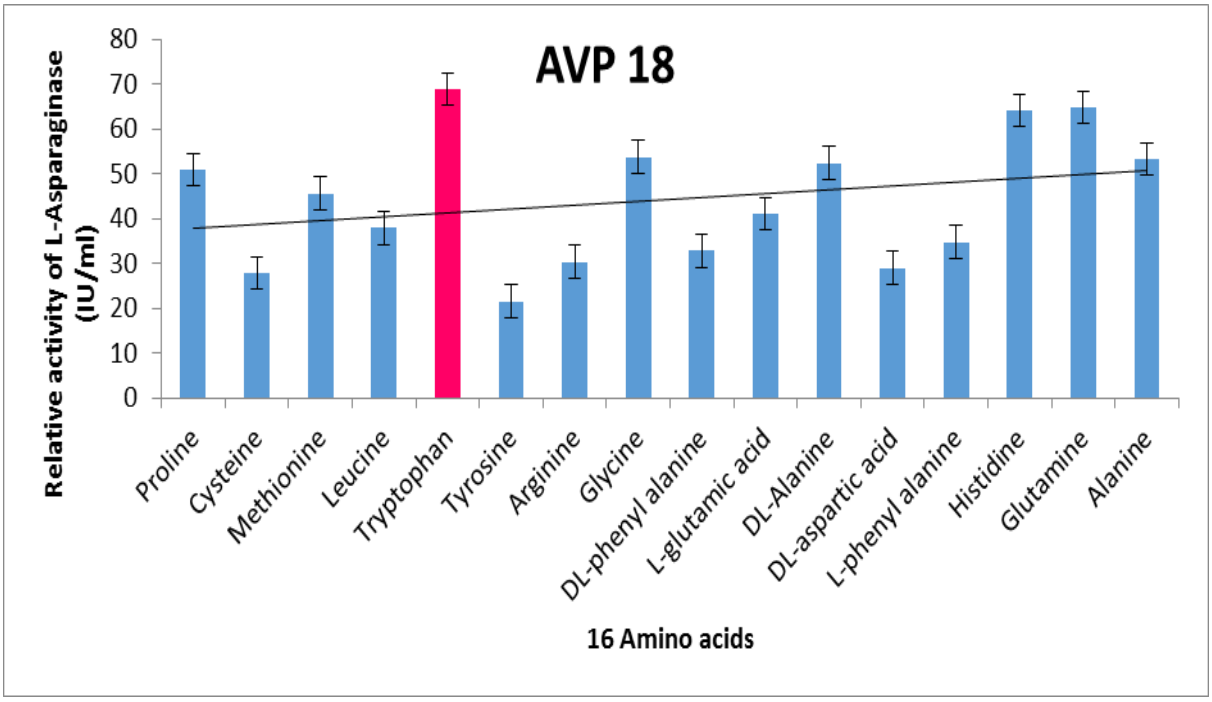


Fig.11 Effect of Metal ions/Mineral salts

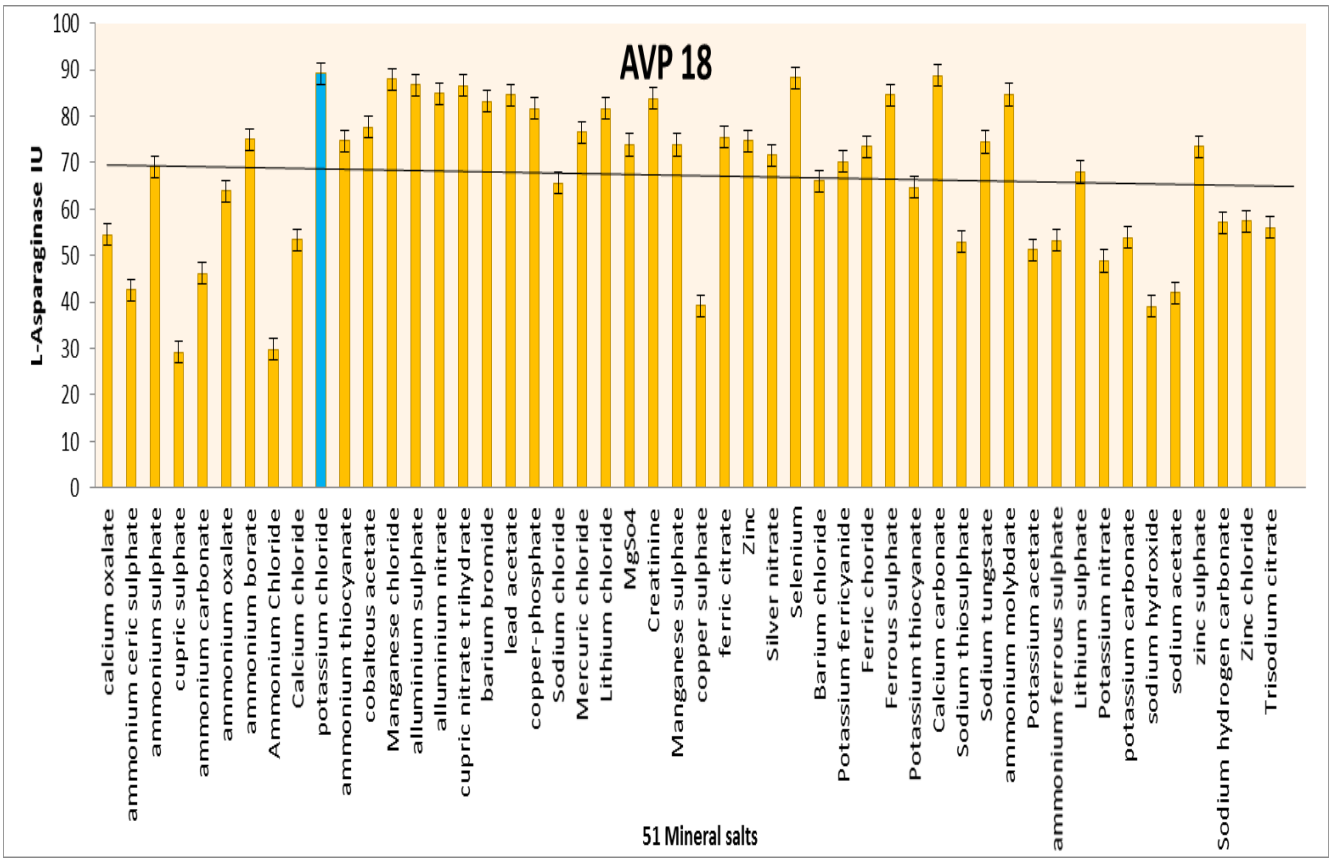
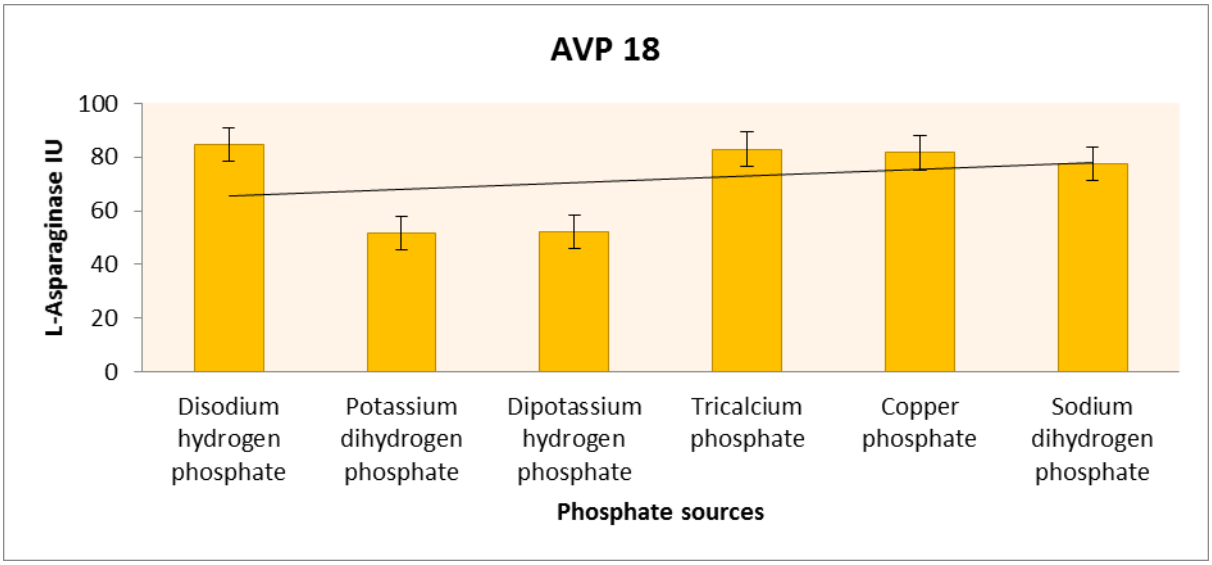


Fig.12 Effect of Phosphate sources



Percentage of NaCl concentration also effect the L-asparaginase production of AVP 18.The production was inversely related with increasing concentration of NaCl and found to be maximum(28.52 IU/ml) at 0.5% concentration(Figure 7).

Isolate AVP 18 showed maximum L-asparaginase production with Dextrose (171.96 IU/ml) (Figure 8). To determine the effect of carbon sources on L-asparaginase yield, different carbon sources were tested which include 21 sugars. Each of them at a concentration of 0.5% w/v with other optimized conditions was supplemented to the production medium of AVP 18 and they have exerted a considerable effect on the biosynthesis of L-asparaginase. Baskar and Renganathan 2011 reported that glucose was found to be best carbon source for maximum L-asparaginase production using modified Czapek-dox media containing soya bean flour as substrate by *Aspergillus terrus* MTCC 1782 Baskar and Renganathan, 2011.

AVP18 found to be produce maximum enzyme production in Beef extract as principle nitrogen source (176.78 IU/ml) (Figure 9). The supplementation of 11nitrogen sources to the production medium had shown a profound impact on the production of L-asparaginase by AVP18.Gaffar and Shethna, 1977 observed the positive effect of supplementation of ammonium sulphate in the production of L-asparaginase Gaffar and Shethna, 1977. Sreenivasulu *et al.*, 2009 have reported ammonium sulphate exhibited maximum enzyme production by the isolated fungus VS-26.

Amino acid Tryptophan (68.9 IU/ml), Mineral source Potassium chloride (89.19 IU/ml) and Phosphate source Di sodium hydrogen phosphate (84.6 IU/ml) were observed to be potential inducers for L-asparaginase production (Figure 10,11 and 12). After optimization Dextrose, Beef extract, Tryptophan, Potassium chloride and Disodium hydrogen phosphate were selected as potential inducers and enhancers.

An attempt was made to evaluate the extent of improvement in production of l-asparaginase in the modified formulated

production media with necessary inducers and enhancers. 5 folds of enhancement in L-asparaginase production observed with modified production medium indicating highly significant improvement so far observed.

L- asparaginase is a clinical acceptable antitumor agent for the effective treatment of lymphosarcoma and lymphoblastic leukemia (ALL). L-asparagines (L-asparagine amino hydrolase) catalyses the hydrolysis of L- asparagine into aspartic acid and ammonia. L- asaraginase is isolated from various sources such as bacteria, yeast, fungi and plant cell. Lasparagines produced by different cultivation process namely solid state fermentation and submerged fermentation. Production of L- asparaginase affected by various physical and chemical parameters such as C and N concentration, pH, temperature. Many purification techniques used for the purification of L-asparaginase. Among the number of treatments acute leukemia such as steroids, intensive combined treatments, radiation therapy, including stem cell transplants or bone marrow chemotherapy is most preferable.

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