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PRODUCTION, PURIFICATION AND CHARACTERISATION OF L-ASPARAGINASE FROM A SOIL ISOLATE *GRIMONTIA HOLLISAE (VIBRIO)*

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ABSTRACT

L-asparaginase is an anti-cancer enzyme used in lymphoblastic leukaemia chemotherapy. Bacterial isolates were screened for potential producers of L-asparaginase using a phenol red indicator in growth medium and those microbial culture displayed pink red coloured colony was selected for further studies and was identified as *Grimontia hollisae (Vibrio)* by using fatty acid methyl ester (FAME) analysis. The enzyme production was carried out by submerged fermentation. The enzyme was partially purified by ammonium sulphate precipitation and dialysis was carried out to remove the excess salt. A Lineweaver-Burk analysis showed a *Km* value of 5.95 mM and *Vmax* of 0.588 IU/min. The characterised enzyme exhibited maximal enzyme activity at pH 8 and temperature 37°C. The activity of L-asparaginase is activated by mono-cations K^+, Na^+ and various effectors including 2-mercaptoethanol, whereas it is moderately inhibited by various divalent ions Hg^{++}, Cu^{++} and Zn^{++}. Substrate specificity studies indicated that, L-asparaginase has greater affinity towards L-asparagine. The amino acid composition of L-asparaginase was also determined.

KEYWORDS: ***L-asparaginase, Amino acid composition, purification, Grimontia hollisae (vibrio), FAME-GC Analysis***

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INTRODUCTION

L-asparaginase (L-asparagine amido hydrolase, E.C. 3.5.1.1) belongs to an amidase group that catalyses the conversion of L-asparagine to L-aspartic acid and ammonium. Asparagine is not an essential amino acid in normal cells and they synthesize this amino acid by the catalytic activity of asparagines synthetase from aspartic acid and glutamine. However, neoplastic cells cannot produce L-asparagine due to the absence of L-asparagine synthetase[1] and they depend on cellular pools of L-asparagine for their growth. Tumor cells, more specifically, lymphatic tumor cells require huge amounts of asparagines for their rapid and malignant growth. L-asparaginase exploits the unusually high requirement tumor cells have for the amino acid asparagine. Discovery of L-asparaginase as a medicinal agent for treatment of cancer was made in 1922. This enzyme has been isolated, purified and experimentally studied in detail as an anti-leukaemia agent in human patients[2,3] and observed its high potential against childhood acute lymphoblastic leukaemia during the induction of remission or the intensification phases of treatment[4,5]. Pegasparginase a pegylated form of the enzyme L-asparaginase derived from *E.coli* is an oncolytic agent used in combination with chemotherapy for the treatment of patients with acute lymphoblastic leukemia who are hypersensitive to native forms of L-asparaginase. The importance of microorganisms as L-asparaginase sources has been focused since the time it was first discovered from *Escherichia coli* and its anti-neoplastic activity demonstrated in guinea pig serum[6-8]. Since then several research groups have extensively involved in isolation of microbial strains such as *Escherichia coli*[9,10], *Erwinia carotovora*[11], *Proteus vulgaris*[12], *Saccharomyces cerevisiae*, *Streptomyces karnatakensis*, *Streptomyces venezuelae* and several fungal genera like *Aspergillus*, *Penicillium* and *Fusarium*[13-15] from various xenobiotic sources producing L-asparaginase enzyme. *Erwinia* asparaginase is considered to be comparably less toxic and is frequently employed in the event of allergic reactions to *Escherichia coli* asparaginase although *Erwinia* asparaginase has a shorter half life than *E.coli* asparaginase[16]. Erwinase®, Kidrolase®, Crisantipase®, Oncospar® and Elspar® are some of the formulations containing L-asparaginase from *Erwinia chrysanthemi* and *E.coli*. In addition, long term administration of enzyme protein produces the corresponding antibody in the living bodies and the antibody causes an anaphylactic shock or neutralization of the drug effect[17]. Therefore, a search for new L-asparaginase immunologically different from that existing has been greatly desired. L-asparaginase production is highly influenced by carbon and nitrogen sources in *Staphylococci* and repressed by L-asparagine and L-aspartic acid while the enzyme production was inhibited by the presence of glutamine and urea in *Aspergillus tamari* and *Aspergillus* terreus[18]. L-asparaginase enzyme activity and its antilymphoblastic leukemia activity from *Grimontia hollisae (Vibrio)* have not been fully explored. L-asparaginase producing strain was screened successfully from diseased potato sample. The strain was further identified as *Grimontia hollisae (Vibrio)* based on its physiological, biochemical characteristics as well as fatty acid methyl ester (FAME) profile. The present investigation deals with isolation, partial purification and optimization of L-asparaginase from *Grimontia hollisae (Vibrio).*

MATERIALS AND METHODS

Chemicals

All the chemicals were purchased from Hi-media Laboratories (India) and were used without further purification unless stated otherwise.

Preliminary screening, selection and maintenance of culture

Infected potato tubers showing typically developed soft rotting were subjected for isolation. Samples of potato tubers rot were collected from local market, nanded. Diseased tubers were firstly washed with tap water then was put into sterilized mortar and homogenized. Screening of bacteria for L-asparaginase production was carried out using rapid assay plate method. A potato samples were serially diluted with sterile distilled water and grown on agar-based modified M9 medium (composition (g/l): KH_2PO_4 2.0, L-asparagine 6.0, $MgSO_4.7H_2O$ 1.0, $CaCl_2.2H_2O$ 1.0, glucose

3.0, and agar 20.0) supplemented with phenol red as indicator[19]. The inoculated agar plates were incubated at 37°C overnight. A microbial culture that displayed pink red coloured colony was selected for further studies. The pinkish red colony (asparaginase-producing bacterial colony) picked from the plates and was streaked on nutrient agar slant. The isolate was grown at 37°C and after growth was stored at 4°C.

Morphological and biochemical characterization of L-asparaginase producing bacterial isolates

Culture was characterised and identified using morphological (Grams stained, observed under light microscope) and biochemical tests (Carbon utilization, Starch hydrolysis, production of H_2S, liquefaction of gelatin, casein hydrolysis, degradation of urea, citrate utilization, indole production, catalase)[20,21].

FAME-GC Analysis

The cultures were obtained from soil sample and pure cultured onto M-9 media at 37°C for 24 hours. The fatty acids are extracted by a procedure which consists of saponification in dilute sodium hydroxide/methanol solution followed by derivatization with dilute hydrochloric acid/methanol solution to give the respective methyl esters (FAMEs). The FAMEs are then extracted from the aqueous phase by the use of an organic solvent and the resulting extract is analyzed by GC. As the bacteria are killed in the saponification step of the extraction, there is little infectivity concern with handling of the sample once this step is concluded. FAMEs are more volatile than their respective fatty acids and therefore more suitable to GC analysis. The Sherlock software automates all analytical operations and uses a sophisticated pattern recognition algorithm to match the unknown FAME profile to the stored library entries for identification[22].

Culture conditions for L-asparaginase production

Fermentations were carried out by inoculating the culture into a liquid M9 medium (composition (g/l): KH_2PO_4 2.0, L-asparagine 6.0, $MgSO_4.7H_2O$ 1.0, $CaCl_2.2H_2O$ 1.0, glucose 3.0, at pH 7). After incubation for 72 hours at 37°C under shake conditions, the media was centrifuged. The supernatant was the source of enzymatic extract and was used for L-asparaginase activity assay.

L-Asparaginase Assay

L-Asparaginase activity was measured by Nessler's reaction[23].The assay procedure is based on direct Nesslerization of ammonia. Enzyme solution (30 µL) was added to Tris–HCl (pH 8.6, 50 mM) in a final volume of 1.5 ml. The reaction was started with addition of 0.5 ml L-asparagine solution (10 mM, in 50 mM Tris–HCl, pH 8.6) and incubation in 37°C water bath for 20 min. The reaction was terminated with addition of 0.5 ml trichloroacetic acid (1.5M) and the volume was adjusted to 4.5 ml with distilled water. Nessler's reagent was added and the tubes were incubated at room temperature for 15 min. After vortexing, the absorbance was measured at 500 nm, using visible spectrophotometer. A standard curve was constructed by taking ammonium sulphate (µM/ml) on x-axis and corresponding optical density on y-axis. One unit (IU) is defined as the amount of enzyme that released 1 µM of ammonia from L-asparagine per min.

Determination of Protein Concentration

Protein of all enzymatic preparations was determined according to Lowry *et al.*[24] using bovine serum albumin as the standard. Readings were carried out in a spectrophotometer at 660 nm.

Optimization of L-asparaginase production

The optimization study of the following parameters was done for better growth and production of the enzyme.

Effect of P^H on L-asparaginase production

The effect of pH on L-asparaginase production from *Grimontia hollisae (Vibrio)* under study was carried out using different pH like 2-9. The optimized media with the above pH were inoculated with culture and the L-asparaginase assay was done after 72hrs. The best pH was concluded by reading the absorbance at 500nm.

Effect of carbon and nitrogen source on L-asparaginase production

Different carbon sources (3 g/l; glucose, lactose, fructose, sucrose and soluble starch) and nitrogen sources (6 g/l; asparagines, beef extract, urea, malt extract, ammonium nitrate

and yeast extract) were chosen to replace glucose and L-asparagine in the production medium. The effect of these different carbon and nitrogen sources on L-asparaginase production under optimal initial P^H and temperature conditions were examined.

Effect of different metabolites on L-asparaginase production

Effect of different metabolites such as Citrate, Pyruvate, Malate, Oxalate and Lactate was studied in concentration 0.5%. In all cases, other previously mentioned optimal conditions were taken into consideration and culture was incubated at 37°C for 72hrs.After incubation period, culture was centrifuged and supernantat was assayed for L-asparaginase activity.

Effect of amino acids and amides on L-asparaginase synthesis

The production medium was used after applying all of the previously mentioned optimal environmental and nutritional conditions for L-asparaginase producing isolate. The used amino acids were added at an equimolecular amount of nitrogen located in the best organic nitrogen source for the enzyme productivity. The supplemented amino acids were phenyl alanine, Therionine, L-Asparagine, Serine, Valine, Histidine, Tyrosine, cystine, methionine, arginine. The optimization media with the above amino acids were inoculated with the culture and the L-asparaginase assay was done after 72hrs. The best amino acid was concluded by reading the absorbance at 500nm.

Purification of L-asparaginase enzyme

Culture filtrate solution was treated with 70% of saturated ammonium sulphate solution. The crude enzyme was continuously stirred using magnetic stirrer and kept at 4°C for overnight, the enzyme was centrifuged at 10,000 rpm for 15 minutes, then supernatant was discarded and the pellets were dissolved in minimum volume of 50mM tris-HCl of P^H 8.6. Dialysis membrane was treated in water bath at 80°C for 10 minutes. Then it was washed thoroughly with glass distilled water. A knot was made at one end the membrane and is used for filling the sample. To this dialysis bag, the sample was added and it was dialyzed against 50mM tris-HCl buffer of P^H 8.6 for overnight at 4°C by placing on a magnetic stirrer. The buffer used for dialysis should be removed twice for the complete removal of salts.

Effect of P^H on catalytic activity of L-asparaginase

The activity of L-asparaginase was evaluated at different P^H values. The purified enzyme was incubated using 0.1 M of four buffers, in the range between P^H 3 - 10, under assay conditions and the amount of ammonia liberated was determined. Buffers used were citrate-phosphate buffer (P^H 3-7), Tris-HCl (P^H 8-9) and glycine-NaOH (P^H 10). The enzyme was incubated for 10 minutes at 37°C at different pH in the presence of substrate L-asparagine and the enzyme activity was determined.

Effect of Temperature on catalytic activity of L-asparaginase

The enzymatic fraction was evaluated at different temperature ranging from 10 to 100°C. Appropriate buffer to maintain optimum P^H of enzyme was used in the system and the enzyme substrate reaction was carried out at different temperatures, velocity of enzyme reaction was measured.

Determination of K_m and V_{max} of L-asparaginase

The Michaelis constant K_m and maximal velocity V_{max} of the purified enzyme were determined using L-asparagine as substrate in the range of 10-100 mM with the help of Lineweaver-Burk plot[25].

Effect of metal ions, thiol compound and chelators on L-asparaginase activity

The effect of metal ions of several mineral salts (i.e. Na^+, K^+, Zn^{2+}, Hg^{2+} and Cu^{2+}) and 2-mercaptoethanol on the enzyme activity was tested at different concentrations (1mM,5mM,10mM). The residual enzyme activity was measured after pre-incubation of L-asparaginase with various chemicals at 37°C for 30 min. L-asparaginase activity measured in the absence of any chemical was taken as control (100%). The remaining L-asparaginase activity was expressed as a percentage of residual activity relative to control. The values represented the mean of 3 replicates and standard errors were reported.

Amino Acid Composition

Amino acid sequences of *Grimontia hollisae (vibrio)* L-asparaginase was retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/) in FASTA format. Amino acid composition of retrieved sequences was done using BIOEDIT software.

RESULTS

Isolation and identification of L-asparaginase producing bacteria

The L-asparaginase positive colonies were screened by formation of pink zone around the colonies of the medium. It indicates deamination with release of ammonia. L-asparaginase activity was confirmed by spot inoculation on L-asparaginase producing medium. As per screening results, the one of the potential isolate was selected for further experimental studies. The bacterium analyzed in the present study was identified based upon the fatty acid composition. The list of the fatty acids composition like straight Chain fatty acids 67.37%, branched chain fatty acid 10.44%, Mono Unsaturated Fatty Acid 6.71% and oleic acid (C18:1) w9c 0.45% was given clearly according to the GC report. The chromatogram obtained in this experimental analysis fig 1 is more descriptive and elaborative. It confirms and correlates the presence of saturated and unsaturated forms of fatty acids present in the bacterium with the predetermined fatty acids unknown fatty acids. Our experimental data matches and establish the similar result mentioned in the report of MIDI Sherlock software databases and the similarity was matched with *Grimontia hollisae (vibrio)*.

Morphological and biochemical characterization of selected bacterial isolate

It is gram negative and rod shaped, slightly curved bacterium, 3.0 – 5.2 μm in length and 0.4 – 0.7 μm in width growing at P^H 7 and temperature 37 °C. It produced oxidase, catalase. (Table 1).Based on morphological, physiological, biochemical characteristics and FAME-GC analysis it was identified as *Grimontia hollisae (Vibrio)* (figure 1).

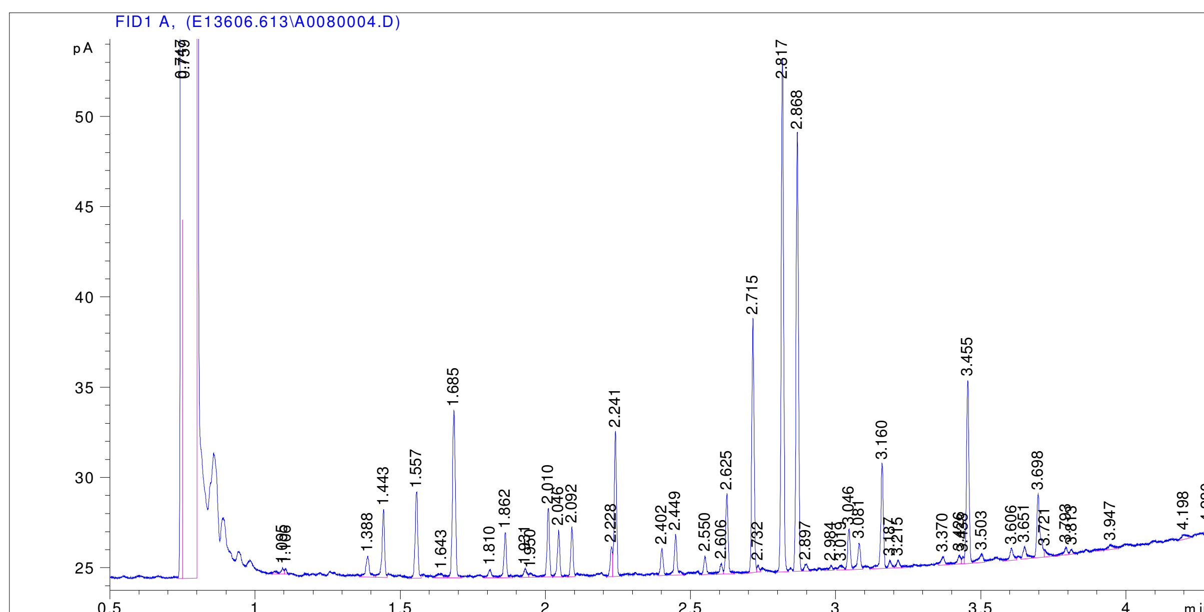

Figure 1
Chromatogram of bacterial sample one showing the fatty acid peaks through Agilent GC 6850.

Characters	Strain
Morphology	Small cells, Rod, slightly curved
Gram nature	-
Motility	+
Oxidase	+
Catalase	+
Urease	-
Nitrate reduction	+
H_2S Production	-
Indole Production	+
Lipase	-
Protease	+
Hydrolysis of:	
Gelatin	-
Utilization of:	
D-Glucose	+
Lactose	-
D-Galactose	+
D-mannose	+

Table 1
Morphological and biochemical characterization of isolated strain

L-asparaginase production conditions
Effect of P^H on the L-asparaginase production
The results presented in Fig.2 indicated a strong influence of P^H on L-asparaginase production .For this study, enzyme yield was compared in a broad range of P^H 2 to 9 in shake flask cultures. The P^H 8 was found to influence L-asparaginase production after three day incubation. Significant level of L-asparaginase production occurs at P^H range 6 to 8.

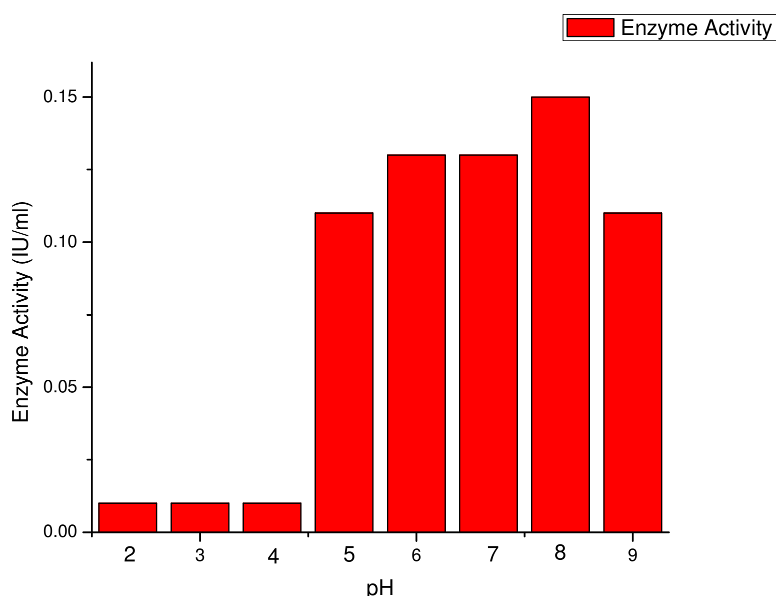

Figure 2
Effect of P^H on L-asparaginase production

Effect of carbon and nitrogen source on L-asparaginase production
Effect of different carbon sources on L-asparaginase production is shown in Fig. 3. Galactose was the best carbon source followed by lactose and starch for L-asparaginase production. Glucose, sucrose dextrose and sorbitol caused a sharp decrease in enzyme

production. With respect to the influence of nitrogen sources on the production of L-asparaginase Fig. 4 shows that based on measurement of enzymatic activity, yeast extract caused remarkably high level of L-asparaginase production, while beef extract, urea and malt extract decreased the production of L-asparaginase. The use of an inorganic nitrogen source, such as ammonium nitrate, also had an effect on L-asparaginase production.

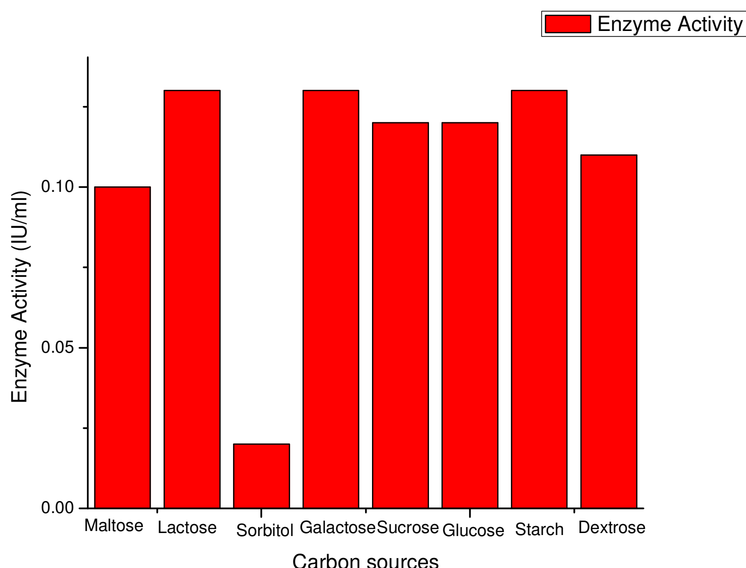

Figure 3
Effect of carbon source on L-asparaginase production

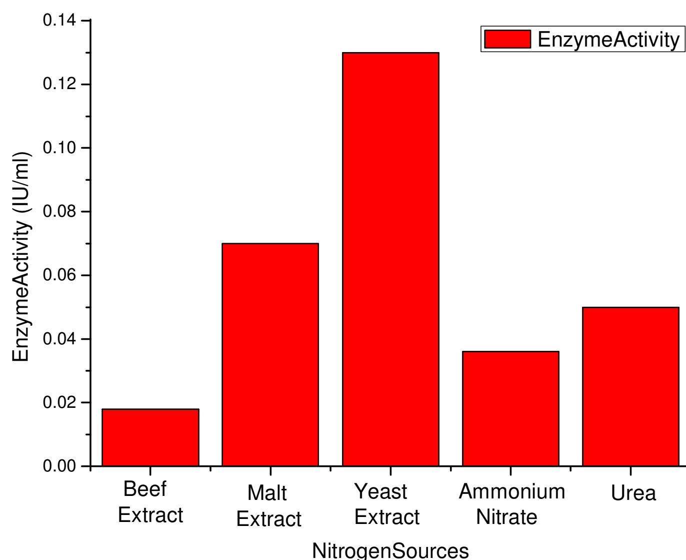

Figure 4
Effect of nitrogen sources on L-asparaginase production

Effect of amino acids and amides

The influence of different amino acids on L-asparaginase production was tested by introducing them in production media. The aim of this experiment was to determine the best amino acid that induces the highest enzyme productivity. The results were revealed that all the tested amino acids exhibited various degrees of L-asparaginase activity but lower than the L-asparagine.

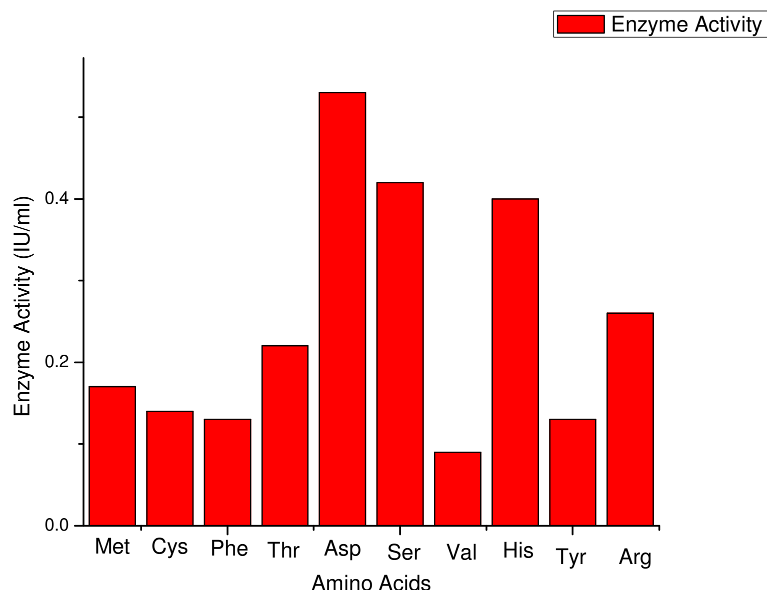

Figure 5
Effect of amino acids and amides on L-asparaginase synthesis

Effect of Different intermediate metabolite
Different intermediate metabolite affects the enzyme activity. Oxalic acid increases the enzyme activity up to 0.14IU/ml (Table no.17) and lactic acid decreases enzyme activity up to 0.04IU/ml.

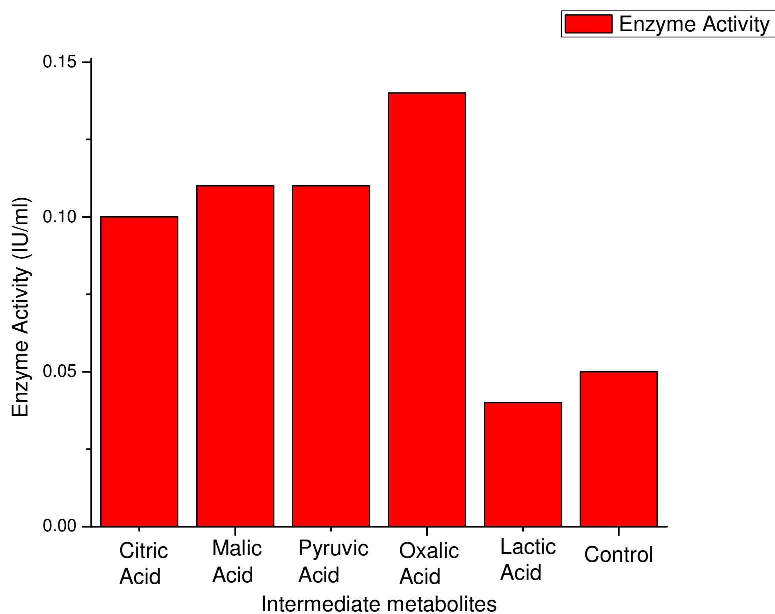

Figure 6
Effect of intermediate metabolites on L-asparaginase production

Purification of L-asparaginase
At the optimized condition, production of L-asparaginase was carried out. The enzyme was purified using 70% ammonium sulphate precipitation and dialyzed against 50mM Tris-HCl of P^H 8.6 for overnight at 4°C. The results of purification steps of L-asparaginase of *Grimontia hollisae (Vibrio)* are presented in Table 2. After the two steps purification the purification fold was 41.

Sample	Enzyme concentration in IU/ml	Protein concentration in mg/ml	Specific activity (IUmg-1)	Purification fold	Recovery (%)
Crude enzyme	0.19	0.07	2.714	1	100
Ammonium precipitation	0.27	0.018	15	5.52	18.11
Dialysis	0.45	0.004	112.5	41	2.43

Table 2
Purification and recovery of L-asparaginase from Grimontia hollisae (Vibrio).

Effect of pH, temperature on the purified L-asparaginase activity

The effect of pH and temperature on the catalytic activity was studied by using L-asparaginase as a substrate under the standard assay condition. The L-asparaginase exhibited maximum activity at pH 8.0 and at 50°C.The pH-stability and thermal stability profile of the L-asparaginase was determined by the measurement of the enzyme activity at various pH values and at various temperatures. The L-asparaginase was stable between pH 6.0 and 8.0 and temperature 20 to 80°C retained activity.

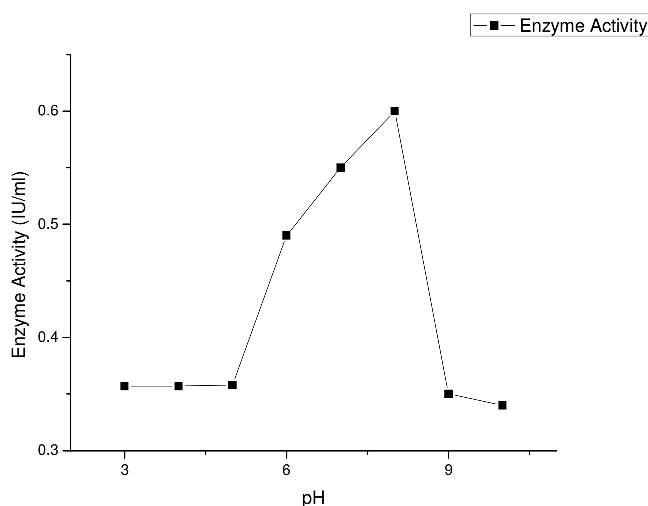

Figure 7
Effect of pH on purified L-asparaginase

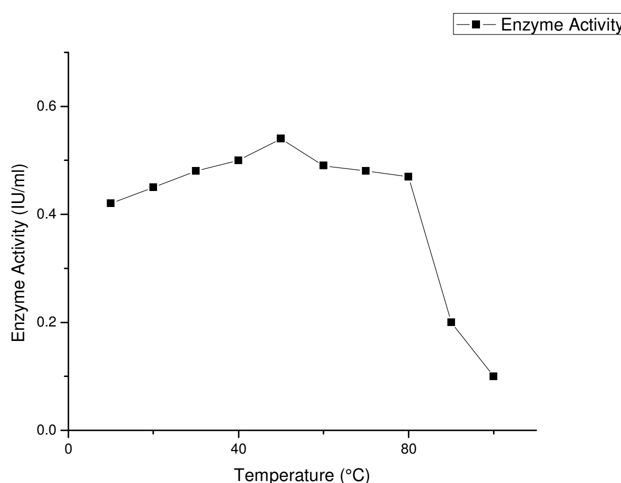

Figure 8
Effect of temperature on purified L-asparaginase

This article can be downloaded from www.ijpbs.net

Effect of different metallic salts and compound on the purified L-asparaginase

The effect of metal ions of several mineral salts (i.e. Na^+, K^+, Zn^{2+}, Hg^{2+} and Cu^{2+}), EDTA and 2- mercaptoethanol on the enzyme activity was tested at different concentrations (1mM,5mM,10mM). After the exposure time, relative enzyme activity in each sample was measured U/ml. Among the salts tested, considerable loss of activity was observed only with Hg^{2+}, Cu^{2+}. However, the highest inhibition value was recorded with Hg^{2+}, which inhibited the enzyme at a final concentration of 10mM, while Na^+, K^+ and mercaptoethanol acting somehow as an enhancer (Table.3).

Activator or Inhibitor	**Relative Activity (%)**		
	10^{-3}M	5×10^{-3}M	10^{-2}M
	100	100	100
Control	116±0.12	127±0.28	148±0.34
NaCl	108±0.21	133±0.28	145±0.14
KCl	88±0.11	122±0.06	204±0.32
2-mercaptoethanol	86±0.31	77±0.36	73±0.27
$CuSO_4$	75±0.22	73±0.23	68±0.28
$ZnSO_4$	65±0.13	40±0.16	25±0.25
$HgCl_2$	90±0.28	75±0.22	60±0.27
EDTA			

Table 3
Effect of different metal cations and compounds on L-asparaginase activity

Determination of K_m and V_{max} of L-asparaginase

Michaelis-menten constants were determined using the optimal reaction conditions in experiments designed to calculate reaction velocities at each substrate concentration. The *K_m and V_{max}* of L-asparaginase towards L-asparagine were determined. The apparent *K_m and V_{max}* of the L-asparaginase for L-asparagine were 6.25 mM and 0.606 U/min, respectively (fig.9).

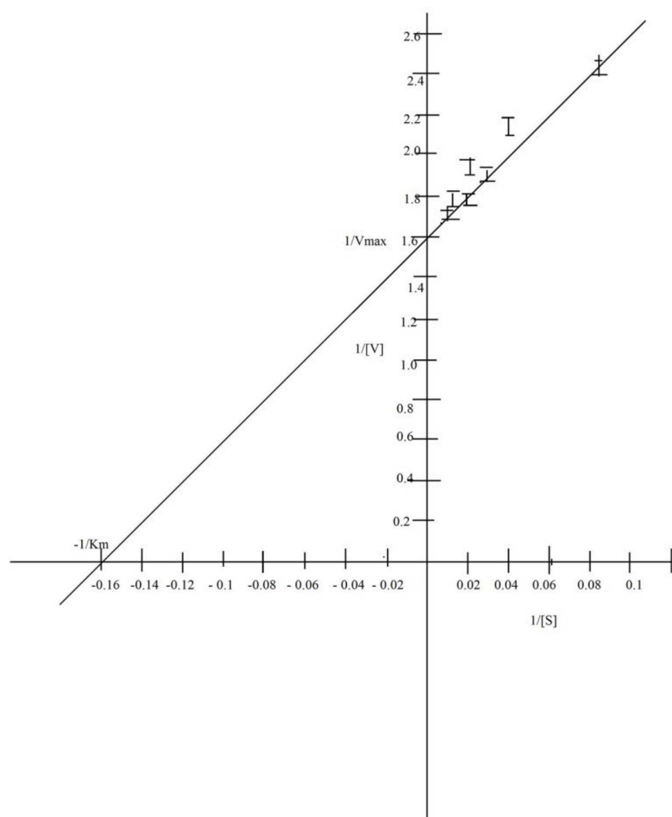

Figure 9
Determination of Km and Vmax of L-asparaginase from Grimontia hollisae (Vibrio). A Lineweaver-Burk plot was used to detect the dependence of L-asparaginase activity on the L-asparagine concentration

Determination of Amino Acid Composition

The primary sequence of the enzyme was retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/). The sequence obtained is given below. MERKHIYIAYTGGTIGMLKSEQGYIPVSGFMQ EQLKQMPEFHRPEMPEFTIHEYSPLIDSSDMT PADWQRIADDIRDNYDNYDGFVILHGTDTMA YTASALSFMLENLDKPVIVTGSQIPLAELRSD GQANLLNALHIAANYPINEVTLFFNNQLLRGN RSTKSHADGFNAFTSPNLPPLLEAGINIQLHG AEIDKKPEGKFKVHTITEQPVAIIMMYPGISPE VIRNALKQPVNAMILLTFGVGNAPQNQELLGL LREATSRGVVVLNLTQCLAGKVNMGGYATG CALAEAGVLSGYDMTPEAALAKLHFLLSQDLP MDALRTQLQQDLRGELTL The molecular weight obtained from BIOEDIT data (336 amino acids) was 36984.38 Daltons and the amino acid composition of L-asparaginase was Ala-Cys-Asp-Glu-Phe-Gly-His-Ile-Lys-Leu-Met-Asn-Pro-Gln-Arg-Ser-Thr-Val- Trp – Tyr. The data shows, L-asperaginase was rich in leucine and alanine.

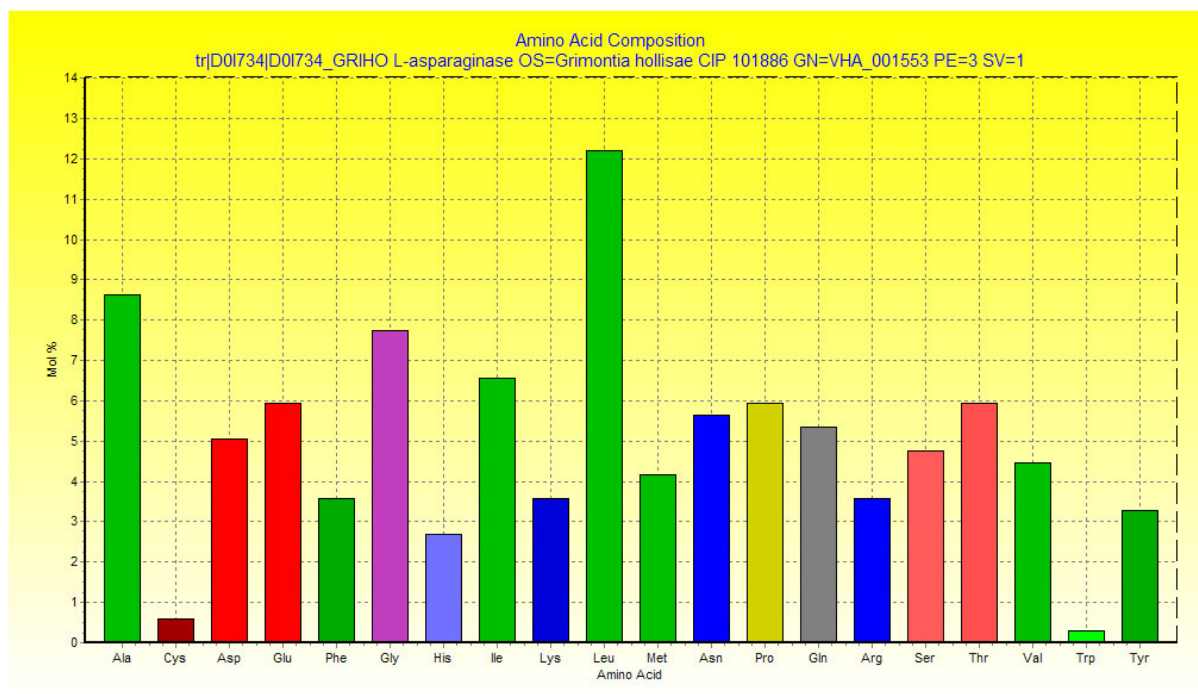

Figure 10
Amino Acid Composition of L-asparaginase from Grimontia hollisae (Vibrio).

DISCUSSION

L-asparaginase, an important therapeutic enzyme has been isolated from a number of sources However, *E.coli* and *E.carotovora* asparaginase has shown anti-tumour activity. In the present study, a bacterial strain producing L-asparaginase enzyme was isolated from diseased potato and identified as *Grimontia hollisae (Vibrio)* based on its morphology, biochemical and FAME-GC analysis. Natural lipids consist of complex mixtures of molecular species, which are found in association with cell membranes, lipoproteins and other sub cellular structures. The composition differs among different cell and tissue types. Fatty acid profiling is a popular method for characterizing microbial communities of natural systems. Direct extraction of microbial fatty acids *in situ* would be useful compared with methods that extracted lipids first and subsequently release fatty acids from lipids. The occurrence and abundance of microbial fatty acids have been used by many investigators for the identification of microorganisms in microbial communities. The Microbial Identification System (MIDI) for fatty acid methyl ester (FAME) analysis is a standard method for identification of microorganisms. Whole cell fatty acids are converted to methyl esters and analyzed by gas chromatography. The fatty acid composition of the unknown is compared to a library of known organisms in order to find the closest match. *Grimontia hollisae (vibrio)* could grow well at 37°C (optimum growth temperature), at pH 8.0.Other workers also reported, L-asparaginase production in different microorganisms[26, 27]. Kinetic study showed that purified L-asparaginase stable under pH 8.0. The K_m of L-asparaginase for L-asparagine was found to be 5.95 mM. Higher K_m value 6.6 and 7.0mM for L-asparaginase from *Lupinus arboreus* and *Lupinus angustifolius* respectively has been reported [28]. On the other hand, a lower K_m value (0.058mM) was obtained for L-asparaginase from *Erwinia chrysanthemi* [29]. The isolate was capable of utilizing a wide variety of carbon sources. Galactose, lactose and starch were the best carbon source in the present study. Boeck et al [30] observed glucose to increase the level of L-asparaginase in *E.coli* and *Pectobacterium carotovora* [31]. Organic nitrogen sources were more efficiently utilized as compared to inorganic sources for enzyme production. The beneficial effect of yeast extract is in agreement with the observation in *Serratia* [32], *E.coli* [33, 34], *Erwinia aeroideae* [35]. Presence of L-asparaginase showed good enzyme productivity as compare to other amino acids. Serine, Histidine was also found to

improve enzyme level. Appreciable stimulatory effects of oxalate, citrate, pyruvate and malate have been found in *Grimontia hollisae (Vibrio).*Lactic acid either has slight inhibitory effect. In *E.coli* [36-38], stimulatory effect of lactate, pyruvate, malate are known. In *Bacillus*[39] oxalate exerts stimulatory effects while citrate, pyruvate were inhibitory. In *Erwinia*[40], L-asparaginase production is stimulated by citrate, malate. This result indicate the high affinity of L-asparaginase towards its natural substrate which proved that L-asparaginase synthesized in *Grimontia hollisae (vibrio)* was an inducible enzyme and may relates its degree of effectiveness against tumors.

CONCLUSION

A systematic study to explore the soil sources of prokaryotes, producing L-asparaginase was carried out. The study included identification of the isolate, optimization of process parameters, and purification of the enzyme. Preliminary screening, secondary screening aiming at identifying the best yielding isolate was selected for developing as source for the current bacterial L-asparaginase. The nitrogen source L-asparagine was found to be primarily responsible for yield of L-asparaginase. The isolate was identified as *Grimontia hollisae (Vibrio)* by morphological, biochemical and FAME-GC Analysis. Enzyme from selected *Grimontia hollisae (Vibrio)* was subjected to purification, characterization and kinetic parameters studies. Purification of the enzyme was carried out sequentially by Ammonium Sulfate precipitation followed by salting out using dialysis. The purity fold of the enzyme after all purification steps were found to be 41. The specific activity of isolate *Grimontia hollisae (Vibrio)* producing L-asparaginase after all purification steps was found to be 112.5 IUmg-1 proteins (Table no.2). The purified sample was found to have optimum activity at pH 8.0 and 40°C. The purified sample was found to be stable at pH range of 6 to 8 and temperature ranging from 20 to 50°C. The enzyme was found to be inhibited by $Hg^{2+}$and Cu^{2+} ions while mercaptethanol, Na^{+}, K^{+} ions were found to enhance the enzyme activity (Table no.3) The K_m and Vmax of the enzyme was found to be 6.25 mM substrate and 0.606 IU/min. The lower K_m value indicates the better substrate specificity**.** The bacterium *Grimontia hollisae (Vibrio)* produced significant amount of L-asparaginase after 72 h of incubation in fermentation medium at 37°C and pH 8. Maximum enzyme production was with lactose, galactose and starch as carbon source, Urea (inorganic nitrogen source). Amino acids exhibited L-asparaginase activity but lower than the asparagine. Intermediate metabolite affects the enzyme activity. Oxalic acid increases the enzyme activity while lactic acid decreases enzyme activity. The above results were encouraging and worth pursuing for further development of the *Grimontia hollisae (Vibrio)* as an alternative resource for therapeutic L-asparaginase. Protein sequencing, gene identification and other bioinformatics parameters help further in establishing the value of the *Grimontia hollisae (Vibrio).*

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