Cloning and molecular analysis of L-asparaginase II gene (ansB)

ABSTRACT

The deamination of L-asparagine to L-aspartic acid and ammonia is catalyzed by L-asparaginases (L-asparagine amino hydrolase). The enzyme L-asparaginase is widely distributed in nature from different living organisms, starting from bacteria till mammals and plants. It has been recently thought to be a therapeutic agent in treatment of various lymphoblastic leukemia diseases. There have been many attempts to isolate microorganisms that produce L-asparaginase. L-ASNase producing bacteria, Escherichia coli MG27, was previously isolated from the River Nile and identified. In this study, ansB gene, encoding L-ASNase II from E. coli MG27, was amplified by PCR, cloned and characterized by DNA sequencing. The DNA sequence was then analyzed using bioinformatics analysis and translated into amino acid sequence. Identification of highly conserved amino acid sequence motifs was conducted by comparison against the InterPro database. Analysis revealed that the protein sequence had a catalytic domain of L-asparaginase type II (IPR004550) that belong to asparaginase/glutaminase family (IPR006034) and has asparaginase/glutaminase conserved site (IPR020827). According to results predicted using PSItPred tool, ansB consists of eight α-helices and 13 β-strands.

Key words: L-asparaginase, cloning, Escherichia coli, leukemia, sequencing

Introduction

The enzyme L-asparaginase is widely distributed in nature from bacteria, yeast, filamentous fungi, mammals and plants. The amino acid sequence of L-asparaginase II was determined by protein sequencing in the seventies and the nucleotides sequence of the ansB gene was reported in 1990. The L-asparaginase II precursor has a 22-residue N-terminal secretory signal peptide which is cleaved between alanine and leucine residues (amino acids 22 and 23, numbering from the initiating methionine residue) to yield mature protein with N-terminal leucine residues. The 22-residue amino acid secretory signal peptide directs the translocation of the protein through the membrane and has a charged N-terminal region, amino acids from 1 to 6, followed by hydrophobic core, amino acids from 7 to 16, and terminated with more polar region, amino acids from 17 to 22. The mature enzyme consists of 326 amino acids located in periplasm as a homo
tetramer and has molecular weight of 141 kDa and its synthesis is 100 to 1000 fold induced in anaerobic cultures. Each one of the four active sites is located between the N and C-terminal domains of two adjacent monomers. Thus, the L-asparaginase II tetramer can be treated as a dimer of dimers. Despite this fact, the active enzyme is always a tetramer (Aung et al., 2000; Kozak et al., 2002; Khushoo et al., 2004).

The production of E. coli L-asparaginase II is regulated by two pleitropic regulatory proteins, the oxygen-sensitive FNR protein, which activates a number of genes during anaerobiosis (Partridge et al., 2008; Tolla & Savageau, 2011; Shan et al., 2012) and the cyclic AMP receptor protein CRP, which controls the initiation of transcription of genes in various catabolic pathways (Beatty et al., 2003; Uppal et al., 2011; Chen et al., 2012; Kraxenberger et al., 2012).

The information from X-ray crystallography and extensive site-directed mutagenesis studies on E. coli asparaginase II, revealed a number of amino acid residues
that are important for catalysis and substrate binding. Among them Thr 12, Tyr 25, Thr 89 and Lys 162 which play a catalytic role, while the positions of Ser 58, Asp 90, Asn 248 and Glu 283 assist substrate binding (Wehner et al., 1994; Palm et al., 1996; Aung et al., 2000).

Many attempts have been made to clone L-asparaginase (ansB) gene from different bacterial and fungal species. Hüser et al. (1999) cloned a class II glutaminase/asparaginase coding gene (ansB) from Pseudomonas fluorescens into pASK-C expression vector that was transformed into E. coli CU1783. The expressed fusion protein with a C-terminal Histag was purified by affinity chromatography on Talon-Spin columns.

DNA fragment coding for L-asparaginase from E. coli AS1.357 was generated using polymerase chain reaction (PCR) technology. It was cloned into the expression vector pBV220 and transformed into E. coli strains JM105, JM109, TG1, DH5α and AS1.357 (Wang et al., 2001). Khushoo et al. (2004) fused the gene coding L-asparaginase II of E. coli to an efficient pelB leader sequence and an N-terminal 6x his tag. The fused gene has been cloned downstream of the T7lac promoter in pET22b expression vector.

Saccharomyces cerevisiae gene coding for the periplasmic L-asparaginase II was cloned into pPIC9 expression vector in-frame with the S. cerevisiae α-factor secretion signal under the control of the AOX1 gene promoter and expressed in the methylotrophic yeast Pichia pastoris (Ferrara et al., 2006). Furthermore, Kotzia & Labrou (2007) reported the cloning and expression of L-asparaginase from Erwinia chrysanthemi 3937 in E. coli BL21 (DE3)pLysS, using PCR®T7/CT-TOPO® expression vector.

Youssef & Al-Omair (2008) reported the cloning of L-asparaginase II gene from E. coli W3110 into pGEX-2T expression vector in-frame with the glutathione S-transferase fusion protein (GST) in E. coli BL21 (DE3) cells. Also, Cappelletti et al. (2008) cloned ansB gene from the pathogenic strain Helicobacter pylori CCUG 17874. The gene was isolated by PCR using specific primers and the PCR product was cloned into pCR2.1-TOPO cloning vector and sequenced.

Vidy et al. (2011) isolated L-asparaginase II-encoding gene (ansB) with excluding the native signal sequence from E. coli MTCC 739 by PCR technique. The 981-bp amplicon was cloned into pET20b expression vector and expressed in E. coli DE3 cells. Vidy & Pandey (2012) isolated L-asparaginase II gene from a moderately thermotolerant bacterium belonging to Enterobacteriaceae by PCR. They used specific primers that were designed in such a way that the native signal sequence was excluded and the mature gene sequence was cloned into pET20b expression vector with a six histidine sequences at the C-terminal end transformed to competent BL21 DE3 cells. Also, Pokrovskaya et al. (2012) cloned ansB gene from Yersinia pseudotuberculosis and constructed a stable inducible expression system that overproduce L-asparaginase in E. coli BL21 (DE3) cells.

This study targeted ansB gene, encoding L-ASNase II from E. coli MG27 amplification by PCR, cloning and characterization by DNA sequencing. The DNA sequence was then analyzed using bioinformatics analysis and translated into amino acid sequence.

Materials and Methods

Amplifying of ansB gene by PCR

Genomic DNA preparation

Genomic DNA was prepared from E. coli MG27 cells (previously isolated from the River Nile and identified) using GeneJET™ Genomic DNA Purification Kit (Thermo Scientific, USA) and according to its protocol. The purified genomic DNA was store at -20°C.

Primers design for amplification of ansB gene

Primers were designed based on the sequence of E. coli K-12 from GenBank accession number M34277 that yielded a single 1047-bp ORF supposed to encode for L-asparaginase II. Primers were designed for amplification of ansB gene by excluding the native signal sequence.

A DNA fragment coding for the predicted mature part of L-asparaginase II (amino acid residues 23-346) was amplified using primers ansB-F (forward, 5'-GGTGGATCCTTACCAATATCACCATTTAG-3') and ansB-R (reverse, 5'-GGGAAGCTTTTGAATGATGAATCTG-3').

 BamHI and HindIII restriction sites (underlined) were incorporated into the primers at their 5' ends, respectively, to facilitate the directional cloning of the structural asparaginase II gene.

Polymerase chain reaction (PCR)

The genomic DNA isolated from E. coli strain MG27 was used as template for the amplification of ansB gene. It was amplified by polymerase chain reaction without its native signal sequence using ansB-F and ansB-R primers.

The PCR reaction was carried out in a total volume of 25μl. The PCR conditions were as follows: initial
denaturation at 94°C for 4 min, denaturation at 95°C for 40 s, annealing at 52°C for 40 s, extension at 72°C for 60 s for 35 cycles, and a final extension at 72°C for 10 min. The PCR product was then analyzed on 1% agarose gel electrophoresis.

**Agarose gel electrophoresis**

For analyzing DNA samples, agarose gel electrophoresis was used. The DNA bands in the gel were visualized using short wave ultraviolet light provided by a transilluminator and photographed.

**Elution and purification of PCR fragments**

To insure high purity of PCR fragments, the amplified DNA bands were eluted and purified form agarose gels using QIAquick Gel Extraction Kit according to manufacturer's protocol.

**TA-cloning of ansB gene**

**Ligation**

The gel purified PCR product was ligated into the pGEM-T Easy vector (Promega crop. Madison, Wi, USA) using T4 DNA ligase provided in the kit according to the manufacturer's instructions. The concentration of the gel purified PCR product was measured prior to ligation. One µl of the resultant recombinant construct was used to transform E. coli JM109 competent cells

**Preparation and Transformation of CaCl₂ competent cells**

For preparation and transformation of competent cells, CaCl₂-treatment was performed (Sambrook & Russel, 2001).

**Screening of positive colonies**

E. coli JM109 colonies harboring the recombinant plasmid were screened for the presence of insert in pGEM-T easy vector by blue/white color selection on plate containing ampicillin, 5-bromo-4-chloro-3-indoly1-β-D-galactosidase (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG). White colonies were randomly selected. The presence of insert was verified by PCR and restriction endonuclease digestion of plasmids isolated from these white colonies.

**Plasmid DNA preparation**

Plasmid DNA was prepared from white colonies of transformed E. coli JM109 cells using QIAprep Spin Miniprep Kit. The purified plasmid DNA was stored at -20°C until used for confirmation of cloning by PCR amplification and restriction digestion.

**Verification tests for the recombinant ansB clones**

**Polymerase chain reaction (PCR) screening**

Plasmid DNA prepared from white clones was used as template for confirmation of transformation using PCR. The presence of ansB gene was checked by PCR using the insert specific primers ansB-F and ansB-R.

The PCR reaction was carried as previously described. Plasmids from blue clones served as negative control. The PCR products were analyzed on 1% agarose gel electrophoresis for selection of right clones.

**Restriction digestion**

Cloning of the ansB gene into pGEM-T Easy vector was confirmed by single and double restriction enzyme digestion of the recombinant plasmid with specific restriction enzymes. Single restriction of the recombinant plasmid was done with BamHI while double restriction was carried out with BamHI and HindIII.

For linearization of plasmids, one microgram of plasmids was digested with FastDigest BamHI restriction enzyme in 20 µl volume with FastDigest buffer. Digestion reactions were incubated at 37°C for 5 minutes. For release the insert, double digestion was carried out using FastDigest BamHI and FastDigest HindIII restriction enzymes with FastDigest buffer. Reactions were incubated at 37°C in heat blocks for 5 minutes, and then electrophoresed into 1% agarose gel. Agarose gels were visualized by ultraviolet transilluminator. The released inserts were eluted and purified form agarose gels using QIAquick Gel Extraction Kit as described before. Gel purified inserts were used for subcloning into pQE-30 expression vector.

**Nucleotide sequencing**

The nucleotide sequence of the insert was determined according to Sanger dideoxy chain-termination method at GATC Biotech (Konstanz, Germany), using M13 forward and reverse primers. The DNA sequence was determined by automated DNA sequencing method using ABI 3730xl sequence analyzer (Applied Biosystems, Foster City, CA, USA). The forward and reverse DNA sequence reads were assembled to obtain the consensus sequence by using DNA Baser Sequence Assembler software v.3.5.3.

**Bioinformatics analysis**

The DNA sequence was analyzed and translated into amino acid sequence using the BioEdit program (Hall, 1999). Restriction analysis was done using CodonCode Aligner (version 3.5.6). ProtParam tool (Gasteiger et al., 2005) was used for computing physicochemical properties that can be deduced from a protein sequence query, such as molecular weight, theoretical pl, amino acid composition, instability index, aliphatic index and grand average of hydropathicity.
Nucleotide and amino acid sequence data were analyzed against all sequences in the GenBank using the basic local alignment search tool (BLAST) (Altschul et al., 1997). Sequence alignments were performed using CLC Main Workbench (version 6.5).

The Conserved Domain Database tool (CDD) (Marchler-Bauerwas et al., 2009) was used to search for proteins with similar domain architecture and superfamilies that show specific sequence matches complementary to the amino acid query sequence. PROSITE tool (De Castro et al., 2006) was used for the detection of known motifs for the classification of a protein into families sharing functional attributes derived from a common ancestor. PROSITE is a database of protein families, domains and associated patterns as well as of signatures and functional sites. INTERPRO tool (Quevillon et al., 2005) was used for classification and characterization of the protein sequence based on consensus sequences between known families, domains and models and the amino acid query sequence. Protein secondary structure of the putative L-asparaginase II was predicted using PSIpred tool (Jones, 1999).

Results

Amplifying of ansB gene by PCR

Amplification of 981-bp fragment was performed using pair of specific primers ansB-F and ansB-R incorporating the sequence for the restriction endonucleases BamHI and HindIII, respectively (Figure 1).

TA-Cloning of ansB into pGEM-T Easy vector

Three white colonies were picked and subjected to confirmation procedures to detect the recombinant clones harboring the putative gene encoding L-ASNase II from local isolate E. coli strain MG27(Figure 2).

Verification tests for the recombinant ansB clones

Three white colonies designated W1, W2 and W3 were picked from LB/ampicillin/IPTG/X-gal plate. These clones were plated on LB-ampicillin plates, as well as being cultured in LB-ampicillin broth for Plasmid DNA minipreps. The presence of insert was confirmed by PCR and restriction digestion.

Polymerase chain reaction (PCR) screening

Figure 3 showed the amplified products of clones W1, W2 and W3 which had the same expected size (981 bp) for PCR product of ansB gene as in positive control (lane 5). Positive control was conducted by using genomic DNA of E. coli MG27 as DNA template. Negative control was conducted by using plasmid from a blue colony as DNA template and showed no band indicating no recombination (lane 4).

Figure 1. Agarose gel electrophoresis of PCR amplification of putative ansB gene amplified from genomic DNA of E. coli MG27. Lane 1, DNA marker; lane 2, PCR amplicon of putative ansB gene. Genomic DNA isolated from E. coli strain MG27 was used as a template for PCR amplification of ansB gene without its native signal sequence using ansB-F and ansB-R specific primers. The PCR product analyzed using 1% agarose gel electrophoresis showed a fragment of the expected size (981 bp) of ansB gene.

Figure 2. Blue/White screening of transformants. LB/ampicillin/IPTG/X-gal plate was plated with 50 µl of transformation mixture and incubated at 37°C for 18-24 h. White colonies represent recombinant clones while blue colonies represent empty clones.
Confirmation of transformation by restriction digestion

Linearized vector in lane 3 showed one band at the expected size (~4 Kb) while two bands were observed in case of double digested vector (lane 4). A DNA fragment about 981 bp was released from the vector backbone (~3.0 kb). Plasmid extracted from the clone W3 was designated pGEM-ansB and subjected to the nucleotide sequence analysis (Figure 4).

Nucleotide sequencing

Nucleotide sequence of the putative ansB gene of E. coli MG27 was submitted to the NCBI database and an accession number KC416966 was assigned while the deduced amino acid sequence was submitted under accession number AGE81914.

Bioinformatics analysis

Results revealed that, sequence of putative ansB consists of 981 bp codes for 326 amino acids (Figure 5). Restriction map of putative ansB gene generated using CodonCode aligner program was represented in Figure 6.

Using ProtParam tool of ExPASy, The results showed that the protein contains 20 amino acids with valine in the highest percentage (10.7%) while tryptophan was the lowest (0.3%) as detailed in Table 1. Other physiochemical properties obtained from ProtParam analysis has been tabulated (Table 2). The instability index of the protein calculated using ProtParam tool was 19.61. The predicted theoretical isoelectric point (pI) value was 5.66 and its molecular weight was estimated to be 34.5788 kDa. The number of negatively charged residues (Asp + Glu) was greater than the number of positively charged residues (Arg + Lys). ProtParam results showed that 33 residues are negatively charged and 30 residues are positively charged. Additionally the grand average of hydropathicity (GRAVY) and aliphatic index were computed to be -0.214 and 84.33, respectively.

Figure 4. Restriction analysis of constructed pGEM-ansB vector. Lane 1, 1 kb DNA marker; lane 2, undigested vector; lane 3, Linearized vector digested with BamHI restriction enzyme; lane 4, vector digested with BamHI and HindIII restriction enzymes. Plasmid DNA prepared from clone W3 was single and double digested and analyzed on 1% agarose gel electrophoresis. Single digestion with BamHI resulted in a linearized vector at the expected size (~4 kb). Two bands were observed when vector was double digested with BamHI and HindIII. Bands in lane 4 represent the expected vector backbone (~3.0 kb) and the released insert (~981 bp).

The deduced amino acid sequence was utilized for similarity search through BLAST at NCBI selecting non-redundant database. BLAST analysis on the deduced amino acid sequence of putative ansB gene from E. coli MG27 showed 100% identity with L-asparaginases II from Shigella sonnei Ss046 (accession number YP_312053.1), Escherichia coli OK1357 (accession number WP_001345951.1) and E. coli MS 79-10 (accession number WP_001012363.1). In
addition, significant homology with 99-87% similarity was found with several bacterial L-asparaginases including L-asparaginase II from *Escherichia coli* KTE66 (accession number WP_001559780.1), *Shigella boydii* 5216-82 (accession number WP_000394146.1), *Citrobacter freundii* (accession number ACC85692.1), *C. youngae* ATCC 29220 (accession number WP_006686371.1), *Salmonella enterica* subsp. *enterica* (accession number WP_000394193.1), *Enterobacter cloacae* SCF1 (accession number WP_003940358.1) and *Serratia marcescens* VGH107 (accession number WP_004928279.1). Multiple sequence alignment was conducted on these sequences using CLC program. Alignment results revealed that several highly conserved domains were extended along ansB sequences.

**Figure 5.** Nucleotide sequence of putative L-asparaginase II gene (ansB) and its deduced amino acid sequence. The sequence extends, 981 nucleotid length and the translation product of the ansB gene was shown below the nucleotide sequence.
Table 1. Amino acid composition of E. coli MG27 putative ansB calculated using the ProParam tool of ExPASy.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Number of residues</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>33</td>
<td>10.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>8</td>
<td>2.5</td>
</tr>
<tr>
<td>Asparagine</td>
<td>24</td>
<td>7.4</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>27</td>
<td>8.3</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>13</td>
<td>4.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6</td>
<td>1.8</td>
</tr>
<tr>
<td>Glycine</td>
<td>28</td>
<td>8.6</td>
</tr>
<tr>
<td>Histidine</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>13</td>
<td>4.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>23</td>
<td>7.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>22</td>
<td>6.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>6</td>
<td>1.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8</td>
<td>2.5</td>
</tr>
<tr>
<td>Proline</td>
<td>13</td>
<td>4.0</td>
</tr>
<tr>
<td>Serine</td>
<td>16</td>
<td>4.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>33</td>
<td>10.1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>12</td>
<td>3.7</td>
</tr>
<tr>
<td>Valine</td>
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<td>10.7</td>
</tr>
<tr>
<td>pyrrolysine</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Selenocysteine</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 2. Physicochemical parameters of E. coli MG27 putative ansB computed using ExPASy's ProtParam tool.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of amino acids</td>
<td>326</td>
</tr>
<tr>
<td>The instability index</td>
<td>19.61</td>
</tr>
<tr>
<td>Theoretical isoelectric point (pI)</td>
<td>5.66</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>34.5788 kDa</td>
</tr>
<tr>
<td>Total number of negatively charged residues (Asp + Glu)</td>
<td>33</td>
</tr>
<tr>
<td>Total number of positively charged residues (Arg + Lys)</td>
<td>30</td>
</tr>
<tr>
<td>Grand average of hydropathicity (GRAVY)</td>
<td>-0.214</td>
</tr>
<tr>
<td>Aliphatic index</td>
<td>84.33</td>
</tr>
</tbody>
</table>

The deduced amino acid sequence of E. coli MG27 ansB gene was scanned for conserved residues by the Conserved Domain Database (CDD). The CDD results revealed that the mature protein contains a conserved domain of L-asparaginase-like superfamily (CDD accession: cl00216) and L-asparaginase-like domain (CDD accession: cd00411) as visualized in Figure 7.
Figure 7. Prosite analysis of the deduced amino acid sequence of putative ansB showing two active domains. Active site signatures are ASN_GLN_ASE_1 (PS00144) and ASN_GLN_ASE_2 (PS00917) at amino acids 6-14 and 82-92, respectively.

Analysis with PROSITE program revealed that L-asparaginase II contains two recognizable structural domains and their locations are marked below (shadowed). The first structural domain (residues 6-14) is located near the N-terminal section while the second structural domain located within residues 82 to 92.

The two asparaginase/glutaminase active site signatures are ASN_GLN_ASE_1 (PS00144) and ASN_GLN_ASE_2 (PS00917) having active site consensus pattern [LIVM]-x-{L}-T-G (2)-T-[IV]-[AGS] and [GA]-x-[LIVM]-x (2)-H-G-T-D-T-[LIVM]. The amino acids 12 and 89 are the active site residues, respectively. The results of the InterPro database are summarized below (Table 3; Figure 8). InterProScan analysis revealed that the protein sequence had a catalytic domain of L-asparaginase type II (IPR004550) that belong to asparaginase/glutaminase family (IPR006034) and has Asparaginase/glutaminase conserved site (IPR020827).

Table 3. Protein signatures and functional domains of ansB protein identified using InterProScan.

<table>
<thead>
<tr>
<th>Source database accession</th>
<th>InterPro accession</th>
<th>Signature ID</th>
<th>Amino acids</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGRFAMS / TIGR00520</td>
<td>IPR004550</td>
<td>asnASE_II</td>
<td>1-326</td>
<td>3.7e-166</td>
</tr>
<tr>
<td>PIRSF / PIRSF001220</td>
<td>IPR006034</td>
<td>L-ASNase_gatD</td>
<td>1-326</td>
<td>1.8e-94</td>
</tr>
<tr>
<td>SUPERFAMILY / SSF53774</td>
<td></td>
<td>Asp/Glutaminase</td>
<td>1-326</td>
<td>4.0e-111</td>
</tr>
<tr>
<td>PROSITE patterns / PS00144</td>
<td>IPR020827</td>
<td>ASN_GLN_ASE_1</td>
<td>6-14</td>
<td>1.0</td>
</tr>
<tr>
<td>PROSITE patterns / PS00917</td>
<td></td>
<td>ASN_GLN_ASE_2</td>
<td>82-92</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Figure 8. Graphical view of InterProScan showing functional domains from the deduced amino acid sequence of L-asparaginase II. Characteristic signatures of L-ASnase II which were found after analysis of the 326 amino acid sequence in the integrative protein signature database InterPro.
The PSIPred database predicted the following secondary structures of the L-asparaginase II from the amino acid sequence as depicted in Figure 9. PSIPred program revealed that ansB consists of eight \( \alpha \)-helices and 13 \( \beta \)-strands. \( \alpha \)nsB was predicted to be formed of approximately 27\% of \( \alpha \)-helices (88 residues) and 20\% of \( \beta \)-strands (63 residues) while random coils represents 53\% (175 residues).

**Discussion**

Studying L-Asparaginase (L-ASNase) has recently gained much attention for its anti-carcinogenic potential. Several authors documented the use of L-ASNase in cancer therapy (Avramis & Panosyan 2005; Narta et al., 2007; Pieters et al., 2011; Tong et al., 2013). Although L-ASNases are present in many plants, mammalian and bacterial species, only the enzymes from *Escherichia coli* and *Erwinia chrysanthemii* have been produced on industrial scale as chemotherapeutics in acute lymphoblastic leukemia. This is due to their high catalytic activity and specificity towards L-asparagine (Müller & Boos 1998; Aghaiypour et al., 2001; Duval et al., 2002). Apart from the therapeutic use, L-ASNase has a potent application in food industry to reduce acrylamide formation in heat-processed products (Friedman & Levin, 2008; Pedreschi et al., 2008; Kukurová et al., 2013).

The mature sequence of ansB gene was amplified from the genomic DNA of a moderately thermotolerant bacterium belonging to Enterobacteriaceae by PCR and the amplicon of ~980 bp was cloned into pET20b vector (Vidya & Pandey, 2012). Furthermore, a putative L-asparaginase gene consisting of 981 bp was amplified by PCR from the *Pyrococcus furiosus* genomic DNA and the PCR product was cloned into a pET14b vector (Bansal et al., 2010). Many investigators cloned L-asparaginase coding genes from various bacteria such as *Pseudomonas fluorescens* (Hüser et al., 1999), *E. coli* (Wang et al., 2001), *Erwinia carotovora* (Kotzia & Labrou, 2005), *E. crysanthesi* (Kotzia & Labrou, 2007), *Yersinia pseudotuberculosis* (Pokrovskaya et al., 2012) and *Bacillus subtilis* (Jia et al., 2013).

In the present study, the clone harboring pGEM-ansB construct was selected for nucleotide sequencing using M13 forward and reverse primers. Nucleotide sequence of the putative ansB gene of *E. coli* MG27 was submitted to the NCBI database and an accession number KC416966 was assigned while the predicted protein sequence was submitted under accession number AGE81914. Based on the instability index of the deduced amino acids predicted by ProtParam.
The grand average of hydropathicity (GRAVY) of the deduced amino acids was computed to be -0.214. This negative value of GRAVY suggests the hydrophilicity of the protein. The aliphatic index of the deduced amino acids predicted using ProtParam tool was 84.33. This high aliphatic index indicates that the protein can be stable within a wide range of temperature.

Based on BLAST analysis, the deduced amino acid sequence of mature L-ASNase II showed 100% identity with L-ASNase II from Escherichia coli OK1357 (WP_001345951.1), E. coli MS 79-10 (WP_0001012363.1). BLAST analysis of the deduced amino acid sequence revealed significant similarity (99-87%) with L-ASNase II of Shigella boydii 5216-82 (WP_000394146.1), Citrobacter freundii (ACC85692.1), C. youngae ATCC 29220 (WP_006686837.1), Salmonella enterica subsp. enterica (WP_000394193.1), Enterobacter cloacae SCF1 (YP_003940358.1) and Serratia marcescens VGH107 (WP_004928279.1).

The amino acid sequence alignment of putative L-ASNase II from E. coli MG27 with L-ASNase II sequences from other 10 strains of bacteria revealed that the sequence of this enzyme is highly conserved especially with Thr-12, Tyr-25, Thr-89, Asp-90, and Lys-162. It was suggested that these residues are essential for reaction the enzymatic activity (Wehner et al., 1994). Thr-12 and Thr-89 are able to act as primary nucleophiles (Harms et al., 1991; Palm et al., 1996).

In the present study, highly conserved amino acid sequence motifs were identified by comparison against the InterPro database. InterProScan analysis revealed that the protein sequence had a catalytic domain of L-asparaginase type II (IPR004550) that belong to asparaginase/glutaminase family (IPR006034) and has Asparaginase/glutaminase conserved site (IPR020827). According to results predicted using PSIpred tool, ansB consists of eight α-helices and 13 β-strands. C-terminal domain (residues 213-326) was predicted to be consisted of four β-strands and four α-helices. These results agree with Swain et al. (1993).

**References**


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