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Cloning and Expression of *AnsZ* Gene Encoding L-Asparaginase from Local *Bacillus subtilis*

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L-asparaginase is an enzyme that catalyzes the hydrolysis of L-asparagine into L-aspartic acid and ammonia. In medical aspect, L-asparaginase especially those came from *E. coli* and *Erwinia chrysanthemi* used as chemotherapy agent of acute lymphoblastic leukemia (ALL). However, new potential organisms possessing L-asparaginase production capacity with a similar therapeutic effect are still required. In *Bacillus subtilis* strain 168, there are two kinds of L-asparaginase gene, *AnsA* and *AnsZ*. The study of the later L-asparaginase (*AnsZ*) has not been conducted intensively. The aim of this study is, first, to isolate this gene of L-asparaginase (*AnsZ*) from local *Bacillus subtilis* and then to express this gene in *Escherichia coli*. Using PCR-cloning method, an open reading frame (ORF) containing 1128 bp was obtained. The ORF has 99% homology with sequence of L-asparaginase from *Bacillus subtilis* Bsn5. The gene then was subcloned into pET 21d (+) with his_6-tag in the C-terminal of the gene product and expressed in *E.coli* BL21. L-asparaginase activity analyses showed that recombinant *E. coli* containing recombinant plasmid with open reading frame (ORF) L-asparaginase (*AnsZ*) from *Bacillus subtilis* had higher activity than that is not containing ORF L-asparaginase (*AnsZ*). Purification with HisPur ™ Ni-NTA Purification Kit increased the specific activity of recombinant L-asparaginase (*AnsZ*) enzyme 29 fold.

Key words: *Bacillus subtilis*, expression, cloning, L-asparaginase (*AnsZ*)

L-asparaginase merupakan enzim yang mengkatalisis hidrolisa L-asparagin menjadi asam aspartat dan amonia. L-asparaginase terutama yang berasal dari *E. coli* dan *Erwinia chrysanthemi* digunakan sebagai agen kemoterapi leukemia lymphoblastic akut (ALL). Akan tetapi, organisme baru yang potensial untuk produksi L-asparaginase dengan efek yang sama sebagai agen terapi masih diperlukan. *Bacillus subtilis* galur 168, mempunyai dua jenis gen L-asparaginase, *AnsA* dan *AnsZ*. Penelitian L-asparaginase (*AnsZ*) belum dilakukan secara intensif. Tujuan penelitian ini adalah mengisolasi gen L-asparaginase (*AnsZ*) dari *Bacillus subtilis* lokal, kemudian mengekspresikan gen ini di *Escherichia coli*. Dengan menggunakan metode PCR-kloning, sebuah kerangka baca terbuka (ORF) yang mengandung 1128 bp diperoleh. ORF ini memiliki 99% homologi dengan urutan DNA L-asparaginase dari *Bacillus subtilis* Bsn5. Gen kemudian disubkloning ke pET 21d (+) dengan His_6-tag di C-terminal dari produk gen dan diekspresikan pada *E.coli* BL21. Hasil analisa menunjukkan bahwa *E. coli* rekombinan yang membawa plasmid rekombinan mengandung ORF L-asparaginase (*AnsZ*) dari *Bacillus subtilis* mempunyai aktivitas yang lebih tinggi daripada yang tidak mengandung *AnsZ*. Pemurnian dengan HisPur ™ Ni-NTA Pemurnian Kit telah meningkatkan aktivitas spesifik L-asparaginase (*AnsZ*) enzim 29 kali lipat.

Kata kunci :*Bacillus subtilis*, ekspresi, kloning, L-asparaginase (*AnsZ*)

L-asparaginase (L-asparagine aminohidrolase, E.C.3.5.1.1) is an enzyme that catalyzes the hydrolysis of L-asparagine into L-aspartic acid and ammonia. It is used as chemotherapy agent for acute lymphoblastic leukemia (ALL) (Borisova *et al*. 2003; Hegazy and Moharam 2010; Soniyamby *et al.* 2011). Many microbial asparaginases have been produced during past few decades. Several of these microbes are *Escherichia coli* (Ghasemi *et al*. 2008; Onishi *et al.* 2011), *Bacillus subtilis* (Shukla and Mandal 2012), *Bacillus circulans* (Prakasham *et al.* 2010), *Serratia marcescens* (Venil *et al*. 2009), *Pseudomonas aerogenes* (Yasser *et al*. 2002), *Pseudomonas florescens* (Al - Mazini 2007), *Streptomyces noursei* (Dharmaraj 2011), *Aspergillus tamari* (Basha *et al*. 2009), *Aspergillus oryzae* (Olempska-Beer 2007; Basha *et al*. 2009), *Aspergillus terreus* (Baskar and Renganathan 2009), *Aspergillus niger* (OLempska-Beer 2008), *Erwinia carotovora* (Deokar *et al*. 2010), *Erwinia aroideae* dan *Proteus vulgaris* (Al - Mazini 2007), and *Fusarium egulseti* (Hosamani and Kaliwal 2011). However, only L-asparaginase from *E. coli* and *Erwinia chrysanthemi* that have been used as anti-cancer drug and sold commercially (Pieters *et al*. 2008).

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The effect of asparaginase on protein synthesis may result in a number of toxicities, including thrombosis, pancreatitis, hyperglycemia, and hepatotoxicity (Earl 2009). Therefore, new serologically different L-asparaginase from new microbial sources with a similar therapeutic effect is highly desirable (Jain *et al*. 2012). From ten species of bacteria surveyed for L-asparaginase activity, *Bacillus subtilis* provided the highest one (Al-Mazini 2007).

Based on the genomic information *B. subtilis* 168 also has two genes encoding L-asparaginase *AnsA* and *AnsZ* (http://genolist.pasteur.fr/subtilist/index.html). Amino acid sequence of *B. subtilis AnsA* similar with type I L-asparaginase from *E. coli* (*EcAI*), whereas L-asparaginase *AnsZ* from *B. subtilis* similar with type II L-asparaginase sequence from *E. coli* (*EcAII*) (Onishi 2011). Therefore, it is important to isolate this *AnsZ* for further study. Fauziah (2012) studied the expression of *AnsA* encoding L-asparaginase under regulation of *B. subtilis* AQ1 *xyn* promoter. However, the study of L-asparaginase (*AnsZ*) has not been studied yet.

In present work, we describe the isolation of the encoding gene of L-asparaginase (*AnsZ*) from the local. *B. subtilis* and expression of this gene in *Escherichia coli*.

MATERIALS AND METHODS

Strain, Plasmids, and Culture Medium. The local isolate identified as *Bacillus circulans*, was purchased from the ITBCC. We reconfirmed the species by conducting sequencing of 16S rDNA, and found this strain was *B. subtilis*. The medium used for growth of *B. subtilis* was the Luria Bertani (LB) medium broth. After incubating at 37 ºC, 150 rpm for 16 h, *B. subtilis* was cultivated and the cells used as source for the genomic DNA. The plasmid pGEM® T-Easy (Promega) was used for TA cloning and pET 21d (+) was used as a vector for expression of the L-asparaginase (*AnsZ*) gene. The *E. coli* DH5α strain and the *E. coli* BL21 star strain were used as a host for L-asparaginase (*AnsZ*) for gene cloning and expression, respectively. *E. coli* DH5α strain and *E. coli* BL21 star strain were cultivated in LB medium. The recombinant *E. coli* colonies were selected from the LB agar plates containing 100 μgmL^{-1} ampicillin at 37 ºC.

Confirmation of Local Species by 16 rDNA and Cloning of the *Bacillus subtilis* L-asparaginase Gene (*AnsZ*). The genomic DNA of *B. subtilis* was isolated using the Genomic DNA Purification Kit (Fermentas) according to the instruction provided by the manufacturer. For confirmation of the species of isolate, the analysis of 16S rRNA gene was conducted. The 16S rRNA gene fragment was amplified by PCR with universal primers 16S-27f (5'-GAGTTTGATCCTGGCTCAG- 3') and 16S-1525r (5'-AGAAAGGAGGTGATCCAGCC- 3'). The PCR was conducted using DNA Taq polymerase (KAPPA, USA) under the following conditions: denaturation at 94 °C for 1 min, annealing 61 °C 35 s, extension 72 °C 2 min for 30 cycles followed by elongation at 72 °C for 5 min using a thermal cycler (Eppendorf, Germany). The sequencing was performed by ABI 3100 DNA sequencer. The DNA sequence was compared to other bacterial 16S rRNA sequences in NCBI GenBank using BLAST program.

The L-asparaginase (*AnsZ*) gene was amplified from the genomic DNA by PCR using forward primer aspz-bcir-f (5'-ATGAAAAAACAACGAATGCTCGTAC-3') and reverse primer aspz-bcir-r (5'-TCAATACTCATTGAAATAAGCTTGG-3'). Primer were designed according to conserved sequences at C and D terminal of the L-asparaginase (*AnsZ*) coding region from *B. subtilis* 168 (http://genolist.pasteur.fr/subtilist/index.html). The reaction PCR was performed under the following condition: initial denaturation of 95 ºC for 3 min, and 30 cycles of 95 ºC for 45 s (denaturation), 60 ºC for 45 s (annealing) and 72 ºC for 2 min (polymerization), followed by the last cycle of polymerization at 72 ºC for 7 min. The PCR fragment was ligated into the pGEM® T-Easy vector and transformed into *E. coli* DH5α competent cells using heat shock method (Sambrook and Russell 2001). Positive recombinant plasmids were selected by the white/blue selection method and verified by restriction enzyme. The plasmid pGEM® T-Easy *AnsZ* was extracted and purified from *E. coli* DH5α cells using Gel/PCR DNA Fragment Extraction Kit (Geneaid). The plasmid pGEM® T-Easy *AnsZ* was then sequenced at Genetica Science Co. Ltd, and the DNA sequence of the L-asparaginase (*AnsZ*) gene obtained was compared with known bacterial gene using BLAST at NCBI (www.ncbi.nih.nlm.gov/BLAST/).

Subcloning of the *B.subtilis* L-asparaginase Gene (*AnsZ*). The L-asparaginase (*AnsZ*) gene was amplified from the recombinant vector pGEM® T-Easy *AnsZ* by PCR using forward primer ansz-nco-f (5'-CATG**CCATGG**GAATGAAAAAACAACGAATGC

TCGTACTTTTTACCGC-3') and reverse primer ansz-sal-r (5'-ACGC**GTCGAC**TCAATGGTGATGGT GATGGTGATACTCATTGAAATAAGCTTGG-3'). The pET 21d (+) vector and PCR fragment was digested with *Nco*I and *Sal*I and then purified with Gel/PCR DNA Fragment Extraction Kit (Geneaid). The target DNA was ligated into the plasmid pET 21d (+) and transformed into *E. coli* DH5α. The positive clones was verified by restriction enzyme digestion and PCR using forward primer T7 promoter and reverse primer T7 terminator besides to using forward primer ansz-nco-f and reverse primer ansz-sal-r. Whereas, verification by digest using *Sfo*I and the positive clones was double digested with *Nde*I and *Pst*I. The positive clone then was retransformed into *E. coli* BL21 star.

Sequence Analysis. Sequence similarity searches were performed in the GenBank Database using the BLAST program. The sequences were multiple aligned by the programe in www.genome.jp.

Identification of the Gene Product. SDS-PAGE was used to examine the expression level of the target protein and evaluate the molecular mass of the protein. L-asparaginase activity was determined by the Nessler method (Imada *et al*. 1973).

Production of L-Asparaginase (*AnsZ*) enzyme. The positive transformants of *E. coli* BL21 star were grown in the LB medium. Ampicillin was added to a concentration of 100 μgmL^{-1}. Cultures were induced for protein production at an OD_{600} of 0.6 by addition of isopropyl-1-tio-β-galactoside (IPTG) to a final concentration of 1 mM and continuous incubation for 4 hours. Uninduced controls were grown the same except no IPTG was added. Enzyme purified with HisPur ™ Ni-NTA Purification Kit (Fermentas).

RESULTS

Cloning of the *Bacillus subtilis* L-asparaginase *AnsZ* Genomic DNA. The 1544 bp length of sequence of 16S rDNA showed 99% identity to *Bacillus subtilis*, and phylogenetic tree analyses showed that this isolate belongs to the group of this species (Fig 1). The accession number for this 16S rRNA sequence is KM115537. PCR amplicons of genomic DNA of the L-asparaginase *AnsZ* encoding gene from *B. subtilis* were cloned. The amplified products were purified and inserted into the pGEM® T-Easy vector. Then, the recombinant plasmids pGEM® T-Easy *AnsZ* were transformed into *E coli* DH5α cells. The target DNA of L-asparaginase *AnsZ* from *B. subtilis* were confirmed by sequence determination. The sequences of the cloned gene DNA of the L-asparaginase *AnsZ* were deposited in GenBank under the accession numbers of KM 096568.

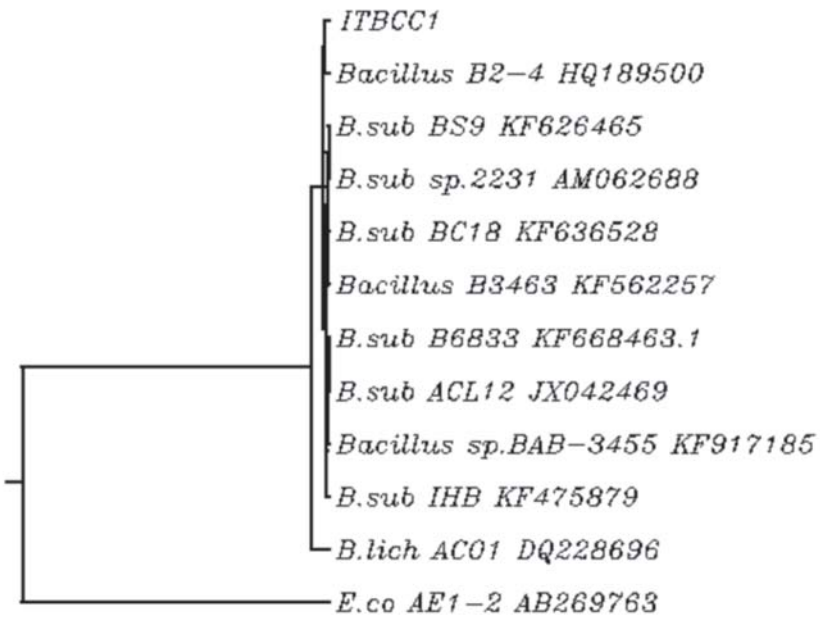

Fig 1 Phylogenetic tree analyses of purchased *Bacillus* sp from ITBCC *Bacillus* sp. strain ITBCC1. *Bacillus* sp. B2-4 (HQ189500), *Bacillus subtilis* isolate p2231 (AM062688), *Bacillus subtilis* subsp. *inaquosorum* strain IHB B 6833 (KF668463.1), *Bacillus* sp. BAB-3455 (KF917185), *Bacillus subtilis* strain BS9 (KF626465), *Bacillus subtilis* strain BC18 (KF636528), *Bacillus* sp. IHB B 3463 (KF562257), *Bacillus subtilis* strain IHB B 4270 (KF475879), *Bacillus subtilis* strain ACL12 (JX042469), *E.coli* AE1-2 (AB269763), *Bacillus licheniformis* ACO1 (DQ228696).

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atgaaaaaacaacgaatgctcgtacttttaccgcactattgtttgtttttaccggatgt
 M  K  K  Q  R  M  L  V  L  F  T  A  L  L  F  V  F  T  G  C
tcacattctcctgaaacaaaagaatccccgaaagaaaaaacgcagacacaaaaagtctct
 S  H  S  P  E  T  K  E  S  P  K  E  K  T  Q  T  Q  K  V  S
tcggcttctgcctctg aaaaaaaaggtctgccaaacattagaattttagcgacaggaggc
 S  A  S  A  S  E  K  K  G  L  P  N  I  R  I  L  A  T  G  G
acgatagctggtgccgatcaatcgaaaacctcaacgactgaatataaagcaggtgttgtc
 T  I  A  G  A  D  Q  S  K  T  S  T  T  E  Y  K  A  G  V  V
ggcgttgaatcactgatcgaggcagttc cagaaatgaaagacattgcaaacgtcagcggc
 G  V  E  S  L  I  E  A  V  P  E  M  K  D  I  A  N  V  S  G
gagcagattgttaacgtcggcagcacaaatattgataataaaatattgctgaagctggcg
 E  Q  I  V  N  V  G  S  T  N  I  D  N  K  I  L  L  K  L  A
aaacgcatcaaccatttgctcgcttcagatgatgtagacg gaatcgtcgtgactcatgga
 K  R  I  N  H  L  L  A  S  D  D  V  D  G  I  V  V  T  H  G
acagatacattggaggaaaccgcttatttttaaatcttaccgtgaaaagtgataaaccg
 T  D  T  L  E  E  T  A  Y  F  L  N  L  T  V  K  S  D  K  P
gttgttattgtcggttcgatgagaccttccacagccatcagcgctgatgggc cttctaac
 V  V  I  V  G  S  M  R  P  S  T  A  I  S  A  D  G  P  S  N
ctgtacaatgcagtgaaagtggcaggtgcccctgaggcaaaagggaaagggacgcttgtt
 L  Y  N  A  V  K  V  A  G  A  P  E  A  K  G  K  G  T  L  V
gttcttaacgaccggattgcctcagcccgatatgtcaccaaaacaaacacaaccacaaca
 V  L  N  D  R  I  A  S  A  R  Y  V  T  K  T  N  T  T  T  T
ggtacatttaaatcagaagaaatgggcttcgtcggaacaattgcagatgatttctatttt
 G  T  F  K  S  E  E  M  G  F  V  G  T  I  A  D  D  F  Y  F
aataatgagattacccgtaagcatacgaaggacacggatttctcggtttctaatcttgat
 N  N  E  I  T  R  K  H  T  K  D  T  D  F  S  V  S  N  L  D
gagctgccgcaggttgacattatctatggataccaaaatgacggaagctacctgtttgac
 E  L  P  Q  V  D  I  I  Y  G  Y  Q  N  D  G  S  Y  L  F  D
gctgctgtaaaagccggagcaaaggggattgtatttgccggttctgggaacgggtcttta
 A  A  V  K  A  G  A  K  G  I  V  F  A  G  S  G  N  G  S  L
tctgacgcagccgaaaaaggggcggacagcgcagtcaaaaaaggcgtcacagtggtgcgc
 S  D  A  A  E  K  G  A  D  S  A  V  K  K  G  V  T  V  V  R
tctacccgcacgggaaatggtgtcgtcacaccaaaccaagactatgcggaaaaggacctg
 S  T  R  T  G  N  G  V  V  T  P  N  Q  D  Y  A  E  K  D  L
ctggcatcgaactctttaaaccccccaaaaagcacggatgttgctgatgcttgcgcttacc
 L  A  S  N  S  L  N  P  Q  K  A  R  M  L  L  M  L  A  L  T
aaaacaaatgatcctcaaaaaatccaagcttatttcaatgagtattga
 K  T  N  D  P  Q  K  I  Q  A  Y  F  N  E  Y  -
```

Fig 2 Sequence and deduced amino acid of the L-asparaginase (*AnsZ*) gene from *Bacillus subtilis*. The signal peptide sequences are underline with bold typeface. The predicted location for signal peptide cleavage. Cleavage site is pedicated between pos 23 and 24: SHS-PE.

Sequence Analysis. The cloned gene DNA sequence was aligned with *Bacillus* sequences in the GeneBank database using BLAST. The homology comparison revealed that the predicted L-asparaginase *AnsZ* gene showed high similarity with L-asparaginase from *B. subtilis* Bsn5 L-asparaginase, complete genome (99% CP002468.1.). The total length of the cloned gene DNA was 1128bp (Fig 2). The deduced amino acid sequence consisted of 375 amino acid residues, with a predicted molecular mass of 40.051 kDa and pI of 6.93. Signal peptide was predicted by SignalP-4.1 prediction. Cleavage site between pos 23 and 24: SHS-PE (Fig 2).

Expression of Recombinant *E. coli*. The *E coli* BL21 star cells containing the pET 21d (+) *AnsZ* were induced with IPTG to a final concentration of 1 mM and incubated as described in the Material and Methods. Cell cultures revealed the presence of a new protein with an approximate molecular weight of 43.3 kDa, as measured by SDS-PAGE, which was not observed in the culture cells of host *E coli* BL21 star cells with the empty pET 21d (+) (Fig 3). The band of the non IPTG sample (lane 5, Fig 3) was not clearly formed compared with IPTG sample (lane 6-8, Fig 3). In figure 3 lane 6-8 shows with band in the box indicating that gene of L-asparaginase (*AnsZ*) has

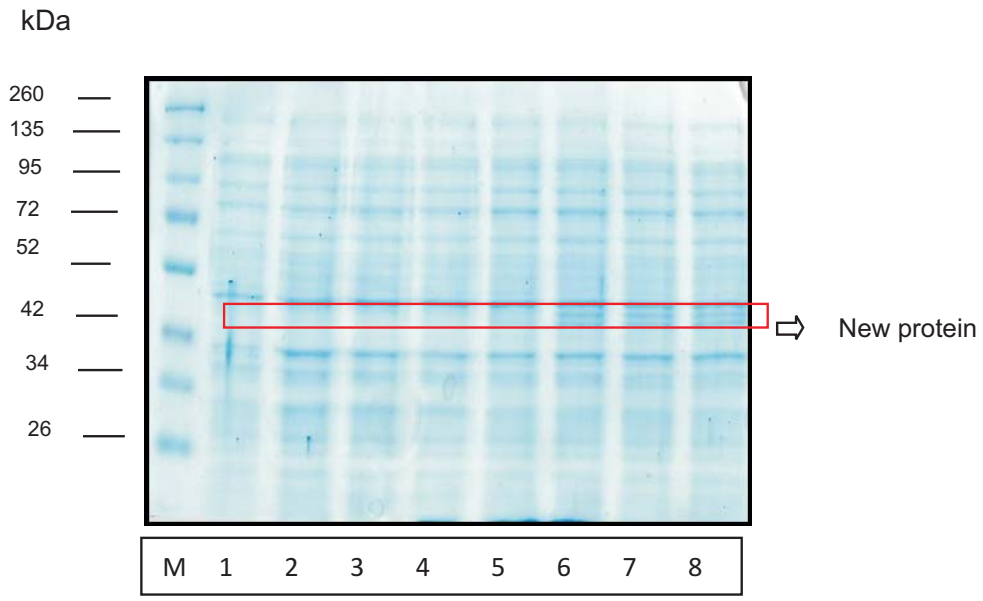

Fig 3 SDS-PAGE analysis of the expressed protein. Lane M, protein molecular marker (*Spectra™ Multicolor Broad Range Protein Leader*). Lane 1, culture cells of *E coli* BL21 star containing pET 21d (+) uninduced by IPTG . Lane 2-4, culture cells of *E coli* BL21 star containing pET 21d (+) induced by IPTG. Lane 5, culture cells of recombianant *E. coli* BL21 star pET 21d (+) *AnsZ* uninduced by IPTG. Lane 6-8, culture cells of recombianant *E. coli* BL21 star pET 21d (+) *AnsZ* induced by IPTG.

expressed via pET 21d (+),whereas, lanes 2-4 contains pET 21d (+) without L-asparaginases (*AnsZ*) gene has no band.

The crude enzyme in the cell exhibited 0.149 µgmL^{-1} of L-asparaginase *AnsZ* specific activity when L-asparagine was used as a substrate. L-asparaginase (*AnsZ*) enzyme activity from *E. coli* containing recombinant plasmid with *open reading frame* (ORF) L-asparaginase (*AnsZ*) from *B. subtilis* was higher than that is containing empty plasmid vector with activity (0.095 µg mL^{-1}). This was showed that ORF L-asparaginase (*AnsZ*) gene was fungsional and expressed in *E. coli*. Purification with HisPur ™ Ni-NTA Purification Kit increased the spesific activity of L-asparaginase (*AnsZ*) enzyme 29 fold.

DISCUSSION

B. subtilis strain 168 has two kinds of asparaginases based on its genome information (Fisher and Wray 2002). The genes was *AnsA* and *AnsZ*. In this study the gene which is encoding asparginase (*AnsZ*) from local *B. subtilis* ITBCC1 has been cloned and expressed. The gene was 1128 bp length, and has 99% identity to those putative asparaginases from *B. subtilis* Bsn5 and *B. subtilis subsp. Subtilis* 6051-HGW.

Prediction of the cleavage site have been performed by a neural network method. SignalP which also performs the discrimination task. SignalP has been available on the word web and mail server is very widely used (Nielsen and Anders 1998; Hiller *et al*. 2004; Hudson *et al*. 2014). The prediction of signal peptide cleavage site is performed at CBS prediction server, SignalP (http://www.cbs.dtu.dk/services/SignalP/) (Tsai 2002; Hudson *et al*. 2014). The SignalP returns three scores (C for raw cleavage site, S for signal peptide, and Y for combined cleavage site) (Tsai 2002). In this study, signal peptide was predicted by SignalP-4.1 prediction. Cleavage site would be between position 23 and 24: SHS-PE (Fig 2).

Specific activity of L-asparaginase (*AnsA*) enzyme used same bacteria was 0.045 µg mL^{-1} (Fauziah 2012); whereas, specific activity of L-asparaginase (*AnsZ)* enzyme from *B. subtilis* in this research (0.149 µg mL^{-1}) (Table 1), higher than L-asparaginase (*AnsA*) enzyme. The difference in specific activity was probably due to characteristic of type I and type II L-asparaginase. The affinity of L-asparaginase type I (*AnsA*) for L-asparagine is lower than L-asparaginase type II (*AnsZ*) (Borek and Jaskolski 2001; Youssef and Al-Omair 2008; Ghasemi *et al*. 2008). Therefore, type II L-asparaginase have been in clinical use in the treatment of acute lymphoblastic leukemia and some other tumors for more than 30 years (Roth *et al*. 2009).

Table 1 Partial purification of L-asparaginase (*AnsZ*) enzyme

Sample	Protein (mg/ml)	Activity (U/ml)	Specific activity (U/mg)	fold
pET 21d (+)	4.947 ± 0.000	0.469	0.095 ± 0.014	-
pET 21d (+) *AnsZ* recombinant	5.593 ± 0.030	0.831	0.149 ± 0.007	1
pET 21d (+) *AnsZ* recombinant purified	0.291 ± 0.000	1.293	4.336 ± 0.046	29.100

Purification with *HisPur* ™ *Ni-NTA Purification Kit* increased the spesific activity of L-asparaginase (*AnsZ*) enzyme 29 fold (Table 1). L-asparaginase (*AnsZ*) enzyme was purified have specific activity 4.336 μgmL^{-1}. In this research, specific activity was lower than specific activity other from *B. subtilis* (Yano *et al*. 2008), *Streptomyces noursei* MTCC 10469 (Dharmaraj 2011), *Fusarium Equiseti* (Hosamani 2011), and *Penicillium* (Soniyamby *et al.* 2011). Further optimization of the production and purification enzyme is needed for improving the enzyme activity. Optimization of growth condition of different agitation, incubation time, and temperature are the method to enhance the L-asparaginase enzyme production. Methods for optimizing purification enzyme are optimizing using imidazole or using different metal ions (Ni^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+}). Higher imidazole concentrations during binding improve the purity; whereas, too high of a concentration decreases the yield. The optimal imidazole concentration during binding is protein dependent (GE Healthcare 2009).

In conclusion, the sequencing result showed that the recombinant plasmids pGEM® - T Easy *AnsZ* had 99% homology with sequence of L-asparaginase from *B. subtilis* Bsn5. L-asparaginase (*AnsZ*) enzyme activity from *E. coli* containing recombinant plasmid with open reading frame (ORF) L-asparaginase (*AnsZ*) from *B. subtilis* was higher than plasmid without ORF L-asparaginase (*AnsZ*). Purification with HisPur ™ Ni-NTA Purification Kit increased the spesific activity of L-asparaginase (*AnsZ*) enzyme 29 fold.

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REFERENCES

Al - Mazini MA. 2007. L-asparaginase activity by various bacteria. Basrah: College of Science.

Basha NS, Rekha R, Komala M, Ruby S. 2009. Production of extracellular anti-leukaemic enzyme L-asparaginase from *Marine actinomycetes* by solidstate and submerged fermentation: purification and characterisation. Trop J Pharm Res. 8(4):353-360. doi: 10.4314/tjpr.v8i4.45230.

Baskar G, Renganathan S. 2009. Statistical screening of process variables for the production of L-asparaginase from cornflour by *Aspergillus terreus* MTCC 1782 in submerged fermentation. Indian J Sci Technol. 2(5):45-48.

Borek D, Jaskolski M. 2001. Sequence analysis of enzymes with asparaginase activity. 48(2):893-902.

Borisova AA, Eldarov MA, Zgoon AA, Alexandrova SA, Omelyn NN, Sokov BN, Berezov TT, Sokolov NN. 2003. Purification and some properties of recombinant *Erwinia carotovora* L-asparaginase, Expresed in *Escherichia coli* cells. Biomeditsinsk aya Khimiya. 49(5):502-507.

Deokar VD, Mangesh D, Rodrignes L. 2010. Production of intracellular L -asparaginase from *Erwinia carotovora* and its statistical optimization using response surface methodology (RSM). Int J Cheml Sci Appl. 1(1):25-36.

Dharmaraj S. 2011. Study of L-asparaginase production by *Streptomyces noursei* MTCC 10469, isolated from marine sponge *Callyspongia diffusa.* Iranian J Biotechnol. 9(2):102-108.

Earl M. 2009. Incidence and management of asparaginase-associated adverse events in patients with acute lymphoblastic leukemia. Clin Advan Hematol & Oncol. 7(9):600-606.

Fauziah A. 2012. Subkloning dan ekspresi gen L-asparaginase dari *Bacillus subtilis* ke *Escherichia coli* DH5α dibawah kontrol promoter *Xyn* AQ1[Subcloning and gene expression of L-asparaginase from *Bacillus subtilis* into *Escherichia coli* DH5α under the control of

xyn AQ1 promoter] [Skripsi]. Depok (ID): Fakultas MIPA, Departemen Biologi, Universitas Indonesia.

Fisher SH, Wray LV Jr. 2002. *Bacillus subtilis* 168 contains two differentially regulated genes encoding L-asparaginase. J Bacteriol. 184(8):2148-2154. doi: 10.1128/JB.184.8.2148-2154.2002.

GE Healthcare. 2009. Recombinant protein purification handbook: principles and methods. Amersham Pharmacia Biotech.

Ghasemi Y, Ebrahiminezhad A, Amini SR, Zarrini G, Ghoshoon MB, Raee MJ, Morow vat MH, Kafilzadeh F, Kazemi A. 2008. An optimized medium for screening of L-asparaginase production by *Escherichia coli.* Am J Biochem Biotechnol. 4(4):422-424. doi: 10.3844/ajbbsp.2008.422.424.

Hegazy WK, Moharam ME. 2010. L-asparaginase hyperproducing recombinant *Bacillus* strains obtained by interspecific protoplast fusion. J Gen Engin Biotechnol. 8(2):*67-74.*

Hiller K, Andreas G, Maurice S, Dieter J. 2004. Predisi: prediction of signal peptides and their cleavage position. Nucleic Acids Res. 32:375-379. doi: 10.1093/nar/gkh378.

Hosamani R, Kaliwal BB. 2011. L-asparaginase an anti tumor agent production by *Fusarium Equiseti* using soniyambi solid state fermentation. Int J Drug Discovery. 3(2):88-99. doi: 10.9735/0975-4423.3.2. 88-99.

Hudson LC, Renu G, Kenneth I, Bost, Kenneth JP. 2014. Soybean seeds: a partical host for the production of functional subunit vaccines. BioMed Res Int. 2014:1-13. doi: 10.1155/2014/340804.

Imada A, Igarasi S, Nakahama K, Isono M. 1973. Asparaginase and Glutaminase Activities of Micro-organisms. J of General Microbiology. 76:85-99. doi: 10.1099/00221287-76-1-85.

Jain R, Zaidi KU, Yogita V, Pooja S. 2012. L-asparaginase: a promising enzyme for treatment of acute lymphoblastic leukiemia. People's J of Sci Res. 5(1):29-35.

Nielsen H, Anders K. 1998. Prediction of signal peptides and signal anchors by a hidden markov model. Proc. Sixth Int: Conf. on Intelegent System for Molecular Biology. 122-130.

Olempska-Beer Z. 2007. Asparaginase from *Aspergillus Oryzae* encoded by the asparaginase gene from *A. Oryzae.* Chem Tech Ass (CTA): 1-7.

Olempska-Beer Z. 2008. Asparaginase from *Aspergillus niger* expressed in *A. niger* . Chem Tech Ass (CTA): 1-7.

Onishi Y, Yano S, Thongsanit J, Takagi K, Yoshimune K, Wakayama M. 2011. Expression in *Escherichia coli* of a gene encoding type II L-asparaginase from *Bacillus subtilis*, and characterization of its unique properties. Annals Microbiol. 61(3):517-524. doi: 10.1007/s13213-010-0167-4.

Pieters R, Appel I, Kuehnel HJ, Fohr IT, Pichlmeier U, Vaart IVD, Visser E, Stigter R. 2008. Pharmacokinetics, pharmacodynamics, efficacy, and safety of a new recombinant asparaginase preparation in children with previously untreated acute lymphoblastic leukemia: A randomized phase 2 clinical trial. J Am Soc Hematol. 112(13): 4832-4838.

Prakasham RS, Hymavathi M, Rao CS, Arepalli SK, Rao JV, Kennady PK, Nasaruddin K, Vijayakumar JB, Sarma PN. 2010. Evaluation of antineoplastic activity of extracellular asparaginase produced by isolated *Bacillus circulans*. *Appl Biochem Biotechnol.* 60:72-80. doi: 10.1007/s12010-009-8679-8.

Roth G, Ev Neves C, Volpato G, Chies JM, Basso LA, Santos DS. 2009. Parameter optimization for recombinant asparaginase production in *Escherichia coli*.

Sambrook J, Russell DW. 2001. Molecular cloning: A laboratory manual, vol 1. 3rd ed. New York: Cold Spring Harbor Laboratory Press.

Shukla S, Mandal SK. 2013. Production optimization of extracellular l-asparaginase through solid- state fermentation by isolated *Bacillus subtilis*. 2013. Int J Appl Biol Pharm Technol. 4 (1):219-226.

Soniyamby AR, Lalitha S, Praveesh BV, Priyadarshini V. 2011. Isolation, production and anti-tumor activity of L-asparaginase of *Penicillium sp*. Int J Microbiol Res. 2(1):38-42.

Tsai CS. 2002. An introduction to computational biochemistry. New York: Wiley-Liss Inc. doi: 10.1002/0471223840

Venil CK, Nanthakumar K, Karthikeyan K, Lakshmana P. 2009. Production of L-asparaginase by *Serratia marcescens* SB08: optimization by response surface methodology. Iranian J Biotechnol. 7(1):10-30.

Yano S, Minato R, Thongsanit J, Tachiki T, Wakayama M. 2008. Overexpression of type I L-asparaginase of *Bacillus subtilis* in *Escherichia coli*, rapid purification and characterisation of recombinant type I L-asparaginase. Annals Microbiol. 58(4):711-716. doi: 10.1007/BF03175579.

Yasser R, Fattah A, Olama ZA. 2002. L-asparaginase production by *Pseudomonas aeruginosa* in solid-state culture: evaluation and optimization of culture conditions using factorial designs. Process Biochem. 38:115-122. doi: 10.1016/S0032-9592(02)00067-5

Youssef MM, Al-Omair MA. 2008. Cloning, purification, characterization and immobilization of L-asparaginase II from *E.coli* W3110. Asian J Biochem. 3(6):337-35. doi: 10.3923/ajb.2008.337.350.