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Purification and Characterization of Thermostable Chitinase from *Bacillus* SW41 for Chitin Oligomer Production

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Bacillus SW41 was isolated from water of shrimp ponds in Maros, South Sulawesi, Indonesia. Molecular identification showed that the isolate belongs to *Bacillus* group, strains SW41. A chitinase produced by *Bacillus* SW41 was purified and characterized. The extracellular enzyme was isolated by successive hydrophobic interaction and gel filtration chromatography. The enzyme was shown to have a relative high molecular weight of 130.2 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Through the stages of purification, chitinase could be purified 8.1 fold. Chitinase SW41 showed optimum activity at pH 4 and stable at 60-70 °C. As a thermostable enzyme, chitinase SW41 showed $t_{1/2}$ relatively long 8.8 h and 8.25 h at 60 and 70 °C, respectively. Another unique feature of chitinase SW41 was indicated on the hydrolysis of colloidal chitin and the ability to produce (GlcNAc)-$(GlcNAc)_5$ through HPLC analysis.

Keywords: Chitinase, *Bacillus*, Oligosaccharides, Chitin.

INTRODUCTION

Chitin is an abundant natural polymer after cellulose, which a major structural component of mollusks, insects, crustaceans, fungi, algae and marine invertebrates [1]. Chitin has a linear β-1,4-linked homopolymer of N-acetylglucosamine, with poor solubility, causing partial application of the compound. The extensive application of polymer chitin is a form of oligomers, which potential functional group contained in the unit of N-acetyl-D-glucosamine. This amine group/acetyl group has primary hydroxy at C2 and C3 as well as secondary hydroxy at C6, which determine the biological properties of chitin [2]. Chitinases can be used to produce various physiologically active oligosaccharides from chitin.

Biodegradation of chitin is performed by chitinases and appears to occur in two steps. An endochitinase (EC 3.2.1.14) degrades the polymer to oligomers, which are subsequently degraded to monomers by exochitinase (β-N-acetylhexosaminidase [EC 3.2.1.52]). Chitinases (EC 3.2.1.14) are a group of enzymes that hydrolyze β-(1,4) chitin linkage. Chitinases are present in a wide range of organisms such as bacteria, fungi, insects, plants and animals [3,4]. Chitinase with different characters have been isolated from *Bacillus thuringiensis* HZP7 [5], *Pseudoalteromonas* sp. DL6 [6], *Thermomyces lanuginosus* SSBP [7], *Brevibacillus formosus* BISR-1 [8], *Citrobacter freundii* str. nov. haritD11 [9], *Bacillus cereus* [10], *Myzus persicae* Aphid [11] and *Alcaligenes faecalis* AU02 [12]. Chitinase's ability to produce oligomers has been based on physiological comprehensive properties of oligomer, which encouraged to find the source of chitinase enzyme to produce chitin oligomers due to the abundant resources [6,7,13-16]. This study was reported about the purification and characterization of chitinase purified from *Bacillus* SW41 as well as application on hydrolysis of chitin. Besides, phylogenetic characterization was also pointed and discussed.

EXPERIMENTAL

$(NH_4)_2SO_4$, K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, yeast extract, tripton, Butyl Sepharose 4 fast flow and Sephadex G-100 were purchased from Sigma-Aldrich Inc. Glycol chitin was prepared from glycol chitosan by the method of Trudel and Asselin [11] with reacetylation using acetic anhydride. Colloidal chitin was prepared from chitin by the method of Arnold and Solomon [17]. All other chemicals and reagents were of analytical grade.

Molecular identification of the SW41 Strain: DNA extraction was performed according to the method of Lazo

and Gabriel [18]. DNA amplification using a kit Ready-To-Go PCR Beads (Pharmacia-Biotech). The 16S rDNA gene was amplified by classical PCR using the universal primers 63f (5′-CAGGCCTAACACATGCAAGTC) &1387r (5′-GGGCGGWGTGTACAAGGC). PCR protocol used is Pre-PCR (94 °C, 2 min), denaturation (92 °C, 30 min), annealing primer (55 °C, 30 min), elongation (75 °C, 1 min) and Post-PCR (75 °C, 5 min) with the number of cycles 30 times. Purification of PCR products was performed using QIAEX II gel extraction kit (Qiagen, Germany). DNA PCR products were visualized by gel electrophoresis and running on the strength of 70 volts of electrical current which is equivalent to 45 mA (Minicicle, Pharmacia Biotech). DNA banding pattern was observed by UV transluminator and recordedby Gel Doc 1000 (Bio-Rad Laboratories Inc.). 16S-rRNA gene was conducted complete sequences in Wilmar Laboratory, Jakarta.

The obtained partial sequences were initially compared with reference sequences by using BLAST (National Centre for Biotechnology Information at (http://www.ncbi.nlm.gov/BLAST) to determine their phylogenetic affiliations. The phylogenetic tree was constructed with Clustal Omega of the Europan Bioinformatics Institute (EBI) and visualized using MEGA 5 software. The confidence level of each branch was tested by bootstrapping 1000 replicates generated with a random seed. The matrix distances were calculated based on UPGMA.

Organism and culture conditions: *Bacillus* SW41was isolated from water of shrimp ponds in Maros, South Sulawesi, Indonesia using agar plates containing colloidal chitin. The obtained single colony was grown in a medium containing chitin with composition: $(NH_4)_2SO_4$ 0.7 %, K_2HPO_4 0.1 %, NaCl 0.1 %, $MgSO_4$ 7 H_2O 0.01 %, yeast extract 0.1 %, tripton 0.1 % dan 1 % colloidal chitin and then incubated at 37 °C for 24 h on a rotary shaker (120 rpm). Ten milliliters of pre-culture was inoculated into a 1.5 L Erlenmeyer flask containing 1000 mL of the same medium and further incubated for 2 days. The culture broth was centrifuged at 4 °C for 20 min at 10000 x g. The supernatant was collected for purification of the enzyme.

Determination of activity and protein content: Measurement of chitinase activity was performed by colorimetric methods using colloidal chitin as substrate [19]. Chitinase activity was assayed in the reaction mixture containing 300 mL of a solution of colloidal chitin enzyme and substrate (0.3 %) in 0.05 M Tris-HCl buffer pH 4. The mixture was incubated at 70 °C for 30 min and then stopped the reaction by boiling at 100 °C for 10 min. Thus, the mixture was centrifuged at 10.000 x g for 10 min to eliminate the remaining chitin. The control was prepared by adding inactivated enzyme after incubation. Subsequently, 2 mL of Schales reagent was added [20] and the clear solution was measured immediately by a spectrophotometer at 420 nm. One unit of activity was defined as the amount of enzyme that liberated 1 μmol of NAG per min during these conditions. Negative control tubes contained all components except substrate and blanks contained all components except the enzyme. The protein content was determined by Bradford method [21] in which BSA was used as a reference protein. Measurement of the enzyme activity and protein content was diluted 10 times.

Precipitation of ammonium sulphate and dialysis: Crude extract of chitinase obtained from the fermentation was used for precipitation of ammonium sulphate.The filtrate of cell-free culture was fractionated with ammonium sulfate with saturation of 50 %. The precipitated protein was obtained by centrifugation at 10.000 x g for 30 min. Protein pellet was dissolved in 0.25 M phosphate buffer (pH 7) and stored at 4 °C. Dialysis was perfomed using a membrane size of 12 kDa cut-off (MWCO) to desalinate the protein extract.

Chromatography: Free-cell supernatant was heated at 80 °C for 5 min and centrifuged at 10.000 x g for 5 min to remove the heat-labile proteins. The supernatant was mixed with $(NH_4)_2SO_4$ to a final concentration of 1 M and the pH was adjusted to 8 with 1 M NaOH. The mixture was loaded on to a butyl sepharose 4-fast flow column (3 × 20 cm) equilibrated with 50 mM phosphate buffer, pH 8 containing 30 % ammo-nium sulphate.

Gel filtration chromatography starting with developing 1 g matrix sephadex G-100 in 100 mL of demineralized water. Warming gel was carried out at 90 °C for 90 min. The matrix was cooled and stored overnight at 4 °C. The matrix was washed twice using Tris-HCl buffer 50 mM, pH 4. Matrix was included in column 2.5 cm × 30 cm. Every 3 mL from the effluent of gel filtration was collected in a fraction and assayed for activity. Protein was measured at 280 nm during chromatographic separation.

Molecular size estimation: The molecular size of the chitinase was estimated by sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [1].

Determination of optimum pH and temperature: The optimal pH and temperature of chitinase SW41 activity were measured using a 10-fold concentrated enzyme. For optimum pH: the chitinase activity was measured at various pHs by a colorimetric method, using colloidal chitin as a substrate. The enzyme was pre-incubated in 50 Mm Tris-HCl buffers (pH 4-5), 50 mM phosphate buffer (pH 6-7) and 50 mM borate buffer (pH 8-9). For temperature optimum: the enzyme was pre-incubated in 50 mM Tris-HCl buffers (pH 4.0) at different temperatures from 40 to 90 °C for 30 min. The reaction was followed by the colorimetric method, with colloidal chitin (0.3 %) as a substrate.

Determination of enzyme stability: Chitinase stability testing at high temperatures was conducted by heating the enzyme at 60, 70 and 80 °C for 1-4 h. Residual activity of chitinase was measured quantitatively using a spectrophotometer. Stability data of thermal was used to determine enzyme deactivation rate constant (k), the half-life of the enzyme ($t_{1/2}$) and the activation energy (E_a).

Preparation of oligomeric chitin: Chitin oligosaccharides assay was done according to the method reported by Wahyuni [4]. Used chitin oligomers are hydrolysis products of chitin by crude extracts and pure chitinase (0.0085 U/mL) of *Bacillus* SW41 isolate. The mixture was incubated for 6 h (crude extract) and 12 h (ammonium sulphate fraction 50 % and Sephadex G-100). Reaction products were analyzed by HPLC to determine the fraction of chitin oligomers produced. HPLC analysis of the hydrolysates chitin oligosaccharides were detected by

high performance liquid chromatography (HPLC) using a Shim-Pack CLC-NH_2 column (Shimadzu Co., Japan). Oligomer analysis was performed in HPLC with an acetonitrile (60 %) mobile phase, a flow of 1 mL/min and a RI detector. GlcNAC n = 1-6 peaks were identified and estimated using a standard calibration curve (1–10 mg/mL) according to Jeon and Kim [22].

RESULTS AND DISCUSSION

Molecular and phylogenetic characterization of chitinase-producing SW41 strain: BLAST analysis of its partial 16S rRNA sequence revealed close matches with members of the genera *Bacillus*. 16S rRNA gene of SW41 isolate was successfully amplified using PCR at 1.35 Kb (Fig. 1). Analysis of SW41 sequence using specific primers was obtained 1222 nucleotides (Fig. 2). Complementary DNA sequences of 16S-rRNA were analyzed with FASTA program from Europan Bioinformatics Institute (EBI) collection.The results of phylogenetic construction with EBI program/MEGA 5 showed that SW41 isolate had 98 % similarity with *Bacillus* sp EU685821. On the basis of the overall taxonomic studies performed, our strain was designated as *Bacillus* SW41 in our culture collection. Construction of the phylogenetic tree was showed in Fig. 3.

Purification of chitinase SW41: SW41 strain was a type of isolates that could produce chitinase. Chitinase SW41 produced chitinase by using colloidal chitin (0.5 %) as an inducer [23]. Fermentation was carried out for 24 h (37 °C and 120 rpm). The produced enzyme was separated from the cells using centrifugation (10000 x g). By this technique, the cells were precipitated due to gravity, while the enzyme remains dissolved in the supernatant. This process was conducted in cold conditions to prevent any damage to the structure of chitinase. The obtained cell-free filtrate had a specific activity of 0.276 U/mg and a protein content of 0.203 mg/mL. Crude enzyme was obtained by precipitation using ammonium sulphate with saturation of 50 %. The use of ammonium sulphate are very effective in maintaining the structure of the enzyme. Precipitation techniques could increase the specific activity of the enzyme of 0.636 U/mg. Various types of chitinase bacteria were successfully purified by ammonium sulphate precipitation pretreatment. *Brevibacillus formosus* BISR-1 and *Bacillus licheniformis* S213 chitinases were precipitated using ammonium sulfate reached saturation of 80 % [8] and 85 % [13], respectively.

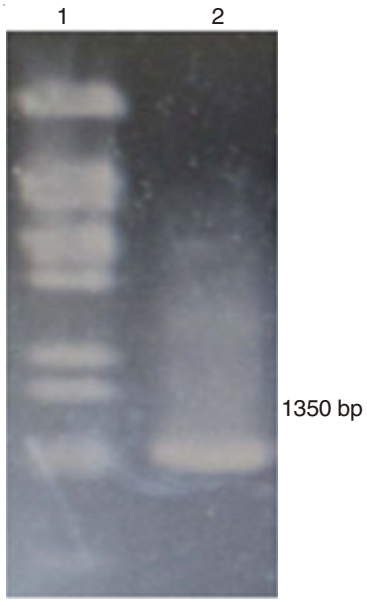

Fig. 1. Electrophoresis analysis of amp lification product of 16S rRNA gene (1) Marker (2) of SW41 strain

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GTATAAAGCTGAAAACCGTGGGTACCTGCCCTAAACTGGGCCCCTCCCTGATCCGGGGCT 1
CTACCGGTAACATTTTGAACCGCATGGTTCGAAATTGACAGGCGGCTTCGCTGTCACTTA 61
TGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATG 121
CGTAGCCGACCTGAGAGGGTGATCGGCCACCCTGGGACTGAGACACGGCCCAGACTCCTA 181
CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCG 241
TGAGTGACGAAGGCTTTCGGGCCACAACACTCTGTTGTTAGGGAAGAACATCTGCTCGTT 301
GCATAAGCTGGCACCTTGACAGAACCTAACCAGAAACCCATTGTTTTCTTTTTGTCTTAG 361
TCTCCTGTGAAGTGTGTGGAAGAAAAGGCCCAACCCGGCTCAACCCGTGGAGGTCATTGA 421
ACTGGGAAGGACCTTGAGTGCAGAAGAGGAAAAGTGGAAATTCCATGTGTAGCGGTGAAA 481
ATGCGTAGAGATATGGAGGAAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGAC 541
ACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA 601
AACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAA 661
GCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG 721
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG 781
ACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTG 901
CATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 961
CTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG 1021
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGC 1081
TACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATATAACCTT 1141
TCTCATTCGGATTGTAGGCTGCAACTCCGCCTACATGAAGCTGAATCGCTAGTAATCGCC 1201
GGATCAGCCTGCCCCCACCACC 1222
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Fig 2. Nucleotide sequences of 16S rRNA gene revealed from SW41 strain

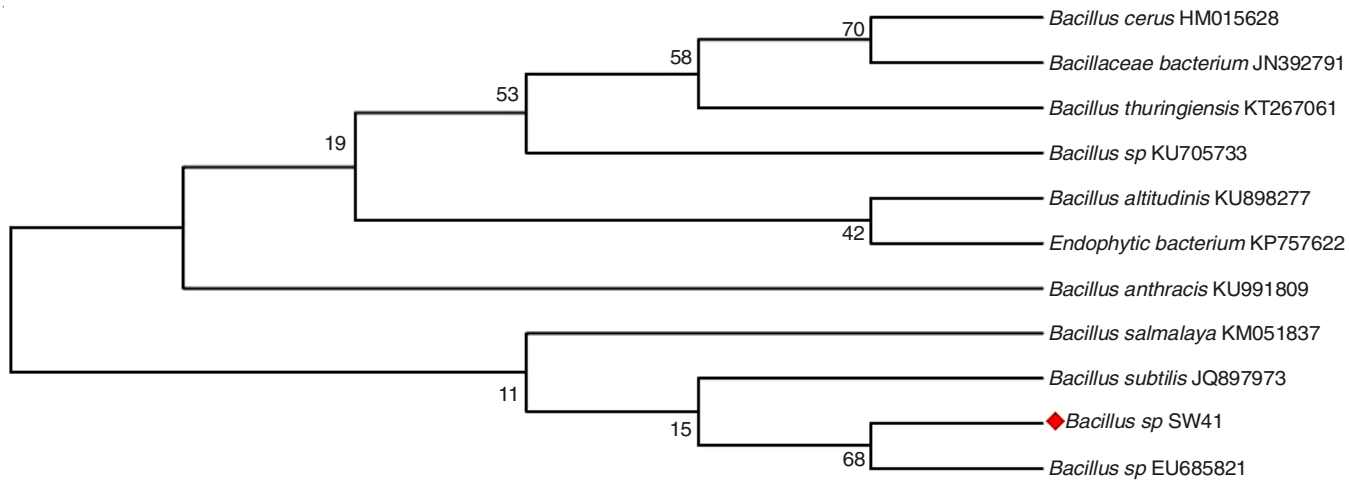

Fig. 3. Phylogenetic correlation based on 16S rRNA gene sequence of SW41 strain. The phylogenetic tree was constructed by using UPGMA method

Further purification was carried out by column chromatography hydrophobic. Before carrying out purification by butyl-sepharose 4-fast flow, the excess of ammonium sulphate in crude enzyme solution was removed by dialysis. Elution profiles of chitinase SW41 with column-butyl sepharose 4-fast flow was shown in Fig. 4. Fraction 29 (F29) was a fraction that showed the highest activity with double protein band. Fraction 29 was further purified using sephadex column G-100 to produce single band on the analysis of sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) (Fig. 5). All stages of the purification of chitinase, *i.e.*, ultrafiltration, ammonium sulfate precipitation and adsorption chromatography were summarized in Table-1.

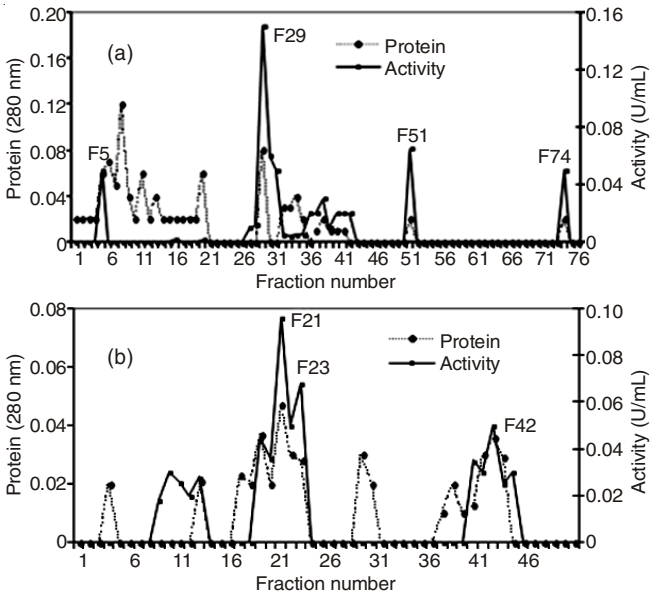

Fig. 4. Chromatogram of purification of chitinase using: (a) Butyl sepharose 4-fast flow column, (b) Sephadex G-100 column

Chitinase was isolated and purified from various species of microorganisms and has been reported by many researchers. Purification of chitinase was carried out in three stages, such as ultrafiltration, ammonium sulfate precipitation at saturation of 50 % and adsorption chromatography using matrix-butyl sepharose 4-fast flow and sephadex G-100. Three peaks were obtained from HPLC using butyl sepharose column, which hadthe highest activity, namely F29, F51 and F74 (Fig. 4A). Through SDS-PAGE control, F29 has two protein bands, thus further separation was needed by using Sephadex G-100 column (Fig. 4B), which was able to separate chitinase SW41 protein with a molecular weight of 130 kDa. Purification stages of chitinase SW41 (Table-1) showed that the purity of the enzyme reached 8 times more pure than crude of chitinase and 5 times from the ammonium sulphate precipitation. The final yield of the purified enzyme was found to be 0.86 %. The specific activity of the enzyme significantly increased

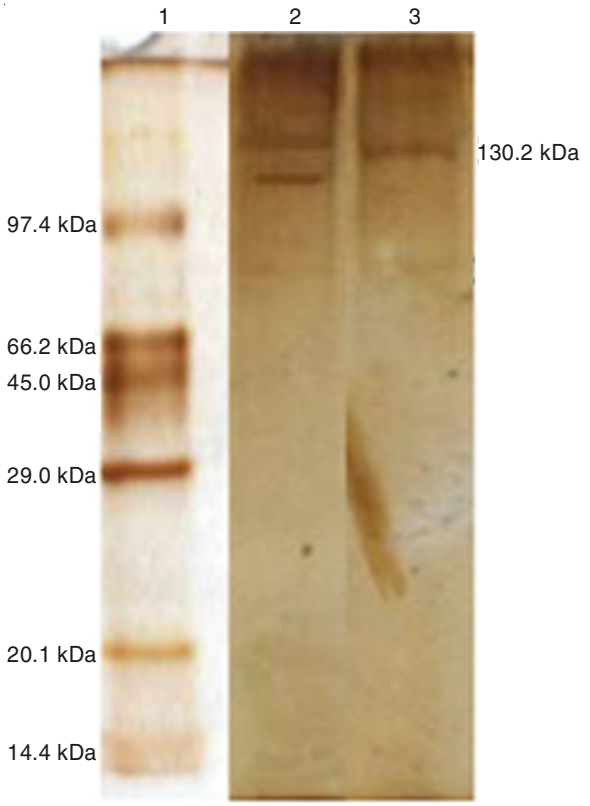

Fig. 5. SDS-PAGE analysis of the purified chitinases. Lane 1 protein marker, Lane 2 F21 (Butyl Sepharose 4-Fast Flow), Lane 3 F21 (Sephadex G-100). Gel was stained with silver staining. The molecular mass markers used for calibration were phosphorylase b (molecular mass, 97.4 kDa), albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

(0276-2233 U/mg) after purified by chromatography adsorption. Toharisman *et al.* [24] reported that purification of chitinase *Bacillus licheniformis* Mb-2 was beginning with hydrophobic columns but further fractionated by ion exchange column and gel filtration obtained chitinase of 67 kDa. Besides, chitinase from *Bacillus licheniform*is S213 had been purified by Slimene *et al.* [13] which has molecular weight of 65 kDa. Chitinase from *Brevibacillus formosus* BISR-1 had molecular weight of 37.6 kDa [8] as well asvarious of chitinase with different molecular weights such as *B. thuringiensis* subsp. colmeri 15A3 (36 kDa) [25], *B. cereus* (36 kDa) [26], *B. circulans* No. 4.1 (45 kDa) [27] and *Bacillus sp.* NCTU2 (36.5 kDa) [28].

Effect of pH and temperature: Chitinase obtained from SW41 strain showed activity at wide pH range (4-10) and temperature (40-90 °C). Chitinase SW41 has optimum activity at pH 4 (Fig. 7), which showed catalytic site of chitinase SW41reaches perfect ionization level. The pH profile of chitinase SW41 indicated that an increase in pH causes a decrease in catalytic activity to hydrolyze colloidal chitin.

TABLE-1
SUMMARY OF PURIFICATION PROFILES OF EXTRACELLULAR CHITINASE FROM *Bacillus* SW41

Purification step	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Culture supernatant	56	0.276	1	100
Ammonium sulphate precipitation (50 %)	1.36	0.636	2.3	2.43
Sephadex G-100	0.48	2.233	8.1	0.86

Several reported chitinase had optimum activity at varying pH such as *Bacillus cereus* (pH 5) [10], *Bacillus licheniformis* Mb-2 (pH 6), *Brevibacillus formosus* BISR-1(pH 5) [8] and *Bacillus licheniformis* S213 (pH 6) [13].

The effect of temperature on the activity of chitinase in SW41 was studied. The optimum temperature for chitinase in SW41 was 60 °C (Fig. 6). Chitinase with the same optimum activity has been reported at *Bacillus thuringiensis* HZP7 expressed in *Escherichia coli* [5] as well as *Pseudo alteromonas* sp. DL-6 [6]. The results of different optimum activity reported in *Citrobacter freundii* harit D11 (35 °C) [9] and *Alcaligenes faecalis* AU02 (40 °C) [12]. Evaluation of thermal stability (Fig. 8) was performed at three different temperatures of 60, 70 and 80 °C. The stability test was conducted for 4 h with intervals of 1 h. At 60 and 70 °C, chitinase SW41 showed stability during 4 h of evaluation, whereas the catalytic activity relative decreased after 2 h of evaluation at 80 °C. For thermal activity test, $t_{1/2}$ chitinase SW41 was determined by drawing natural logarithm of catalytic activity *versus* incubation time. Chitinase SW41 has $t_{1/2}$ 8.8, 8.25 and 2.8 h at incubation temperature of 60, 70 and 80 °C, respectively. The results showed that increasing on incubation temperature, obtained decreasing $t_{1/2}$ chitinase SW41. However, the $t_{1/2}$ of pure chitinase SW41 wasvery different from that of crude chitinase SW41. Activity of crude chitinase SW41 at 60, 70 and 80 °C had $t_{1/2}$ of 115, 6.8 and 12.8 h, respectively (data not shown).

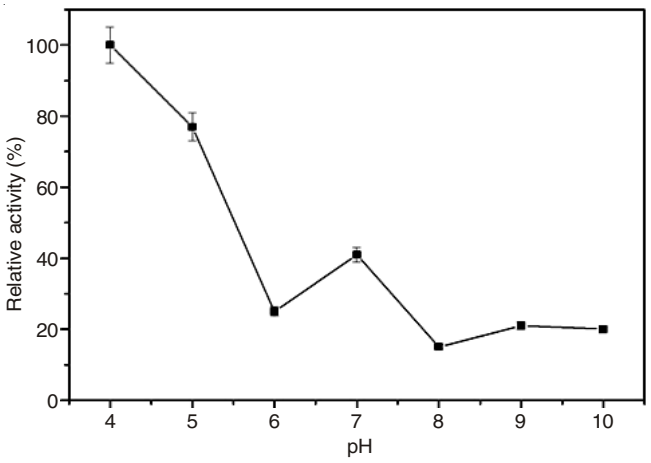

Fig. 6. Effect of pH on enzyme chitinase SW41 activity

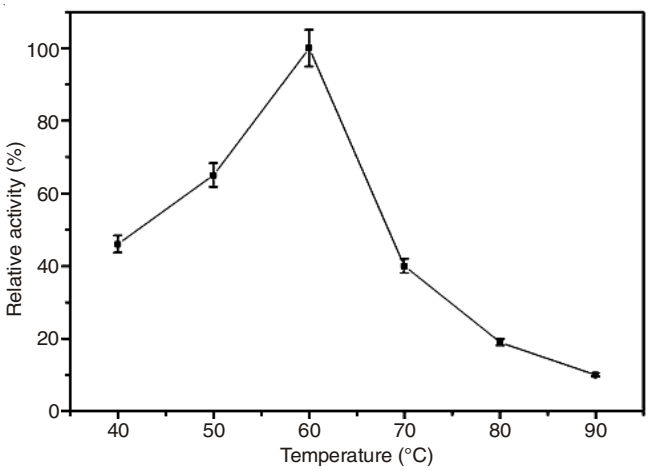

Fig. 7. Effect of temperature on enzyme chitinase SW41 activity

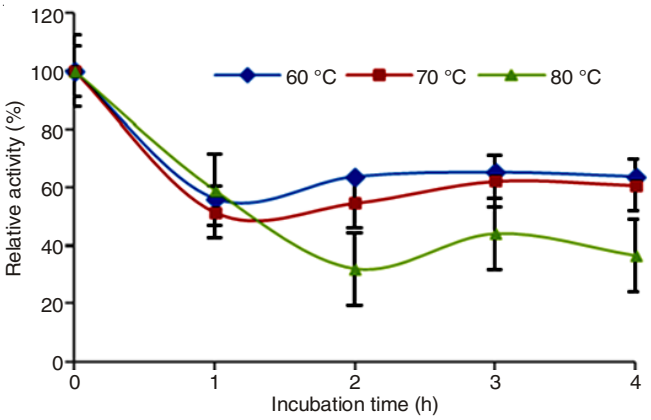

Fig. 8. Effect of temperature on thermal stability

Loss of protein during the purification process may cause the change of conformation stability, which thereby enzyme was not resistant to heat. Calculation of $t_{1/2}$ expressed the stability of the enzyme against heat, which thermostable factors of enzymes stabilizing consist of hydrogen bonding, hydrophobic bonding, ionic interactions and disulfide bridges [29]. The damage bond can increase entropy of enzyme conformations, thus the tertiary structure of the enzyme is more easily denatured [2]. The difference in half-life ($t_{1/2}$) of the enzymehas been reported in chitinase *Paenibacillus* sp. BISR-047 (4 h at 100 °C) [30] and *Brevibacillus formosus* BISR-1 (5 h at 100 °C) [8]. Although crude chitinase SW41 have a longer half-life, but the greater energy was required to hydrolyze colloidal chitin of 26.05 kcal/(gmol K), whereas pure chitinase of 13 kcal/(gmol K). Determination of E_a was involved Arrhenius equation. The slopeof the regression equation of the relationship ln k to the heating temperature (1/T) expressed as E_a value of R (gas constant) (Fig. 9). The uniqueness of the characteristics of chitinase SW41 which exhibits termotoleran, provides application opportunities in the industrial basis enzymes at high temperatures. One potential application is the production of oligosaccharides.

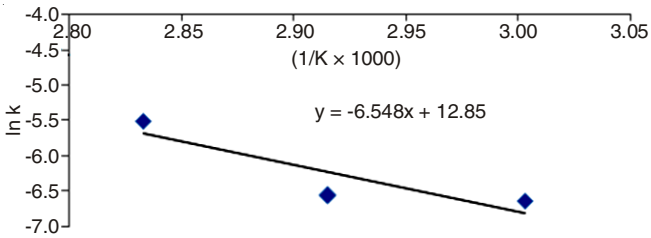

Fig. 9. Arrhenius plot for the determination of E_a for colloidal chitin hydrolysis

Hydrolysis of colloidal chitin: Chitin is a polymer composed of units of N-acetyl glucosamine. Chitinase has ability to hydrolyze chitin to produce monomer units and forms oligomers. Chitin oligomers is a unit of the dimer to pentamer. Hydrolysis of chitin using a cell-free filtrate, ammonium sulphate fractions and fractions Sephadex G-100 was investigated. As a result of optimization, the cell-free filtrate was reacted with a substrate colloidal chitin for 6 h of incubation, whereas the fraction of ammonium sulfate and Sephadex G-100 were incubated with substrate for 12 h. Reaction control of colloidal chitin hydrolysis was performed by using HPLC.

Fraction of the cell-free filtrate, ammonium sulfate and Sephadex G-100 had specific activity of 0.0085 U/mg. Hydrolysis of colloidal chitin enzyme produced 3 fractions monomer (GlcNAc). GlcNAc fraction of ammonium sulphate produced the highest of 1240 ppm (Fig. 10). $(GlcNAc)_2$ dimer was produced by the 3 fractions chitinase SW41, which the smallest hydrolyzate. Ammonium sulphate fraction produced most $(GlcNAc)_2$ of 442 ppm. However, for the trimer $(GlcNAc)_3$, the fraction of ammonium sulphate produced 1500 ppm lower than hydrolysates produced by chitinase cell-free filtrate and Sephadex G-100 fractions. Tetramer $(GlcNAc)_4$ and pentamer $(GlcNAc)_5$ was produced only by ammonium sulphate and Sephadex G-100 fractions. The ability of chitinase SW41 was very different fromexo-chitinase (ChiC) from *Pseudo alteromonas* sp. DL-6 [6]. Hydrolysis of colloidal chitin and $(GlcNAc)_6$ using the Exo-chitinase (ChiC) *Pseudo alteromonas* sp. DL-6 only produced $(GlcNAc)_2$ in all of incubation time for 0 min to 24 h. The different results were also reported in Chi I and Chi II of *Salinivibrio costicola* expressed in *Escherichia coli*, which produced the majority of $(GlcNAc)_3$ on the hydrolysis of colloidal chitin [31] and chitinase *Aeromonas* sp. DYU-Too7 produced $(GlcNAc)_2$ at 5 min of incubation [14]. The different results were also reported on hydrolysis of colloidal chitin used chitinase *Aeromonas* sp. 10S-24 [15] and *A. caviae* [16]. The ability of chitinase SW41 was very different to chitinase from other microorganisms, providing wide application in industrial oligosaccharides.

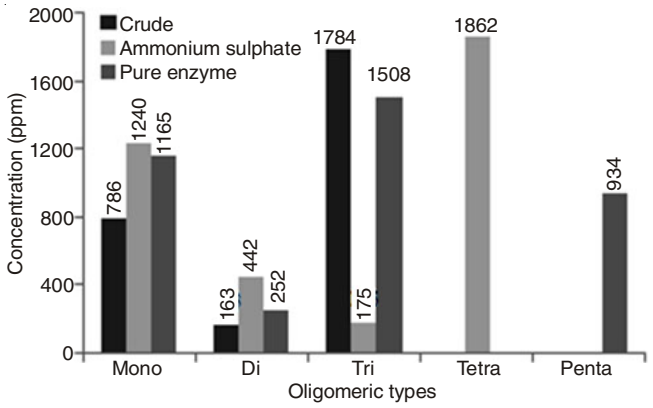

Fig. 10. Concentration of chitin oligomeric from chitinase SW41 hydrolysis

Conclusion

In this study, purification and characterization of chitinase from *Bacillus* strain SW41 had been performed. Obtained enzyme fractions at all stages of purification were used for hydrolysis of chitin colloidal. Chitinase SW41 was successfully purified using hydrophobic and gel filtration column. Determination of molecular weight chitinase SW41 was conducted by using SDS-PAGE. Based on thermal characterization, the chitinase SW41 is thermostable. The ability of chitinase SW41 on hydrolysis of colloidal chitin was very different from the chitinase produced by different types of bacteria. Chitinase SW41 has good potential application in oligosaccarides industry which hasability to produce di, tri, tetra and penta oligosaccharides.

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