academicJournals

Vol. 8(4), pp. 348-354, 22 January, 2014
DOI: 10.5897/AJMR2013.6500
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Production, purification and characterization of cellulase from *Streptomyces* sp.

Mahmoud Abdul-Megead Yassien[1]*, Asif Ahmad Mohammad Jiman-Fatani[2] and Hani Zakaria Asfour[2]

[1]Department of Natural Products/Microbiology, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia.
[2]Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia.

Accepted 27 December, 2013

High cellulase producing *Streptomyces* strain C188 was isolated from a Saudi Arabia soil sample and identified as *Streptomyces longispororuber* by 16S rDNA sequencing. Enzyme productivity by this strain in carboxymethyl cellulase liquid medium reached 8540 U/L after 96 h of incubation at 30°C. Cellulase productivity in tested strain was improved (25084 U/L) by supplementation of the carboxymethyl cellulase liquid medium with 1% corn steep liquor and pH 6.5 (maintained throughout the incubation period using 0.05 M phosphate buffer). Purification of cellulase enzyme was carried out by ammonium sulfate precipitation, diethylaminoethyl cellulose and Sephadex G-75 gel filtration chromatography. The final preparation had 13.5% activity recovery and approximately 38.5-fold purification. The purified enzyme migrated in a single band with molecular weight of 42 kDa on SDS-PAGE. Maximum enzymatic activity was observed at pH 6-6.5 and 50°C, while maximum stability was obtained at pH 6.5 and up to 60°C.

Key words: Cellulase, *Streptomyces longispororuber*, purification.

INTRODUCTION

Cellulase is one of the most important industrial enzymes. Cellulases have attracted interest because of their diversity of applications. In 2001, the world market for enzymes was over $1.5 billion; this was doubled by the year 2008. The United States and Europe each consume 30% of the world output of enzymes. Approximately 75% of industrial enzymes are used for hydrolysis and depolymerization of complex natural substances (Kirk et al., 2002).

Major industrial applications of cellulases are in the textile industry for "biopolishing" of fabrics such as production of the stonewashed look of denims, and in household laundry detergents to improve fabric softness and brightness. Moreover, they are used in animal feeds to improve nutritional quality and digestibility, in processing of fruit juices, and in baking; de-inking of paper is yet another emerging application (Ponnambalam et al., 2011).

In addition, cellulase enzymes are involved in enzymatic hydrolysis of cellulose, one of the most abundant organic materials that can be converted to products with significant commercial interest. Bioconversion of cellulose to monomeric sugars has been intensively studied as researchers seek to produce bioethanol and bio-based products, food and animal feeds, and many valuable chemicals (Barros et al., 2010).

Cellulase enzymes are produced from plant, animal and microbial sources. For commercial production, microbial enzymes have the enormous advantage of being scalable to high-capacity production by established fermentation techniques (Tahtamouni et al., 2006). A wide variety of microorganisms are known for their ability to

*Corresponding author. E-mail: myassien61@yahoo.com. Tel: 00966-508319951. Fax: 00966012-6951696.

Abbreviations: CMC, Carboxymethyl cellulase; **CSL,** corn steep liquor.

produce cellulase enzymes. *Streptomyces* sp. is one of the best known enzyme producers (El-Sersy et al., 2010)

This study focused on isolation of a high cellulase-producing *Streptomyces* strain from soil samples collected in Saudi Arabia, and improvement of enzyme productivity by supplementation with organic nitrogen sources and pH optimization. The enzyme was purified and characterized.

MATERIALS AND METHODS

Isolation of *Streptomyces* isolate

A high cellulase-producing *Streptomyces* strain C188 was isolated from a soil sample in Saudi Arabia as described by Jaradat et al. (2008). The strain was purified by streak-plating on starch-nitrate agar (20 g soluble starch, 2.0 g KNO_3, 1.0 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 3.0 g $CaCO_3$, 0.01 g $FeSO_4$, 0.01 g $MnCl_2$, 0.01 g $ZnSo_4$, 20 g agar per liter).

Growth condition and enzyme production

Enzyme productivity by the tested strain was determined as described by El-Sersy et al. (2010). In brief, a spore suspension from 4 to 5 days old cultures was prepared in normal saline (0.85% NaCl solution) and used as an inoculum for a final count of 10^3 CFU/mL in 50 mL carboxymethyl cellulose (CMC) liquid medium (1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.01 g $MnSO_4 \cdot 7H_2O$, 0.3 g NH_4NO_3, 10.0 g CMC per liter, pH 7) in 250 mL Erlenmeyer flasks and incubated in a rotary shaking-incubator (S19R-2, Sheldon Mfg, USA) at 250 rpm and 30°C for 96 h. After incubation, a known volume of culture broth was centrifuged at 12,000 × *g* for 20 min and the cell pellet was washed twice with distilled water and dried in hot air oven at 100°C to a constant weight. The dry cell weight per liter of culture broth was used to determine microbial growth. The activity of cellulase enzyme in the culture filtrate was determined as described in the procedure below.

Enzyme assay

Cellulase activity was quantified according to Miller (1959). A reaction mixture composed of 0.2 mL crude enzyme solution and 1.8 mL 0.5% CMC in 50 mM sodium phosphate buffer (pH 7.0) was incubated at 50°C in a shaking water bath (GFL 1083, Germany) for 30 min. The reaction was terminated by adding 3 mL 3,5-dinitrosalicylic acid reagent (Sigma Aldrich, USA). The color was developed by boiling the mixture for 5 min. Optical densities were measured at 575 nm by using spectrophotometer (Labomed UVD-3200, UK) against a blank containing all the reagents minus the crude enzyme. Results were interpreted in terms of enzyme activity in which one unit (U) of enzyme activity was defined as the amount of enzyme that liberates 1 µmol glucose per minute under the above assay conditions.

Genetic identification of the selected *Streptomyces* isolate by 16S rDNA analysis

Isolation of the whole genomic of the selected strain was carried out as described by Pospiech and Neumann (1995). PCR amplification of the 16S rDNA gene was conducted using 2 primers, StrepF: 5-ACGTGTGCAGCCCAAGACA-3 and StrepR: 5-ACAAGCCCTGGAAACGGGGT-3 (Edwards et al., 1989). The PCR mixture contained 30 pmol each primer, 100 ng chromosomal DNA, 200 µM dNTPs and 2.5 units Taq polymerase in 50 µL polymerase buffer. Amplification was conducted in an automated thermocycler (Techne, TC-5000, UK) for 30 cycles of 1 min at 94°C, 1 min annealing at 53°C and 2 min extension at 72°C. Amplified products were analyzed by agarose gel electrophoresis and the remaining mixture was purified using QIA quick PCR purification reagents (Qiagen, USA). The 16S rDNA gene was sequenced on both strands via the dideoxy chain termination method (Sanger et al., 1977) with a Terminator Cycle Sequencing kit (Applied Biosystems 3500 Genetic Analyzer, Applied Biosystems, USA). The BLAST search program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to assess the degree of DNA similarity and sequence alignment. Sequence alignment was performed and the neighbor joining phylogenetic tree was constructed using the TREE VIEW program.

Factors affecting cellulase production

The effect of organic nitrogen source on growth and cellulase production was investigated by supplementing the CMC fermentation medium with different organic nitrogen sources and inoculating with 0.5 mL of the spore suspension (10^5 CFU/mL). After incubation, the supernatant was assayed for cellulase activity. Microbial growth was quantified by dry cell weight determination.

To study the effect of initial pH, a set of flasks containing CMC broth with initial pH values from 5.0 to 9.0 was prepared and inoculated with the test strain. To determine the effect of pH maintenance over the incubation period, the ingredients of the CMC medium were dissolved in 0.05 M phosphate buffer at varying pH values. At the end of the incubation period, microbial growth and enzyme activity were evaluated as described above.

Purification of the cellulase enzyme

The strain was cultivated in modified CMC broth at 30°C for 4 days. The microbial culture was centrifuged (12000 × *g* for 30 min at 4°C); the supernatant was collected and the enzyme was precipitated with ammonium sulfate salt (pH 5). The precipitated fraction of the enzyme between 30 and 70% ammonium sulfate saturation was collected by centrifugation (10000 × *g*, 20 min, 4°C), then dissolved in 0.02 M sodium acetate buffer (pH 4.8) and dialyzed against the same buffer. The prepared solution, a crude enzyme preparation, was applied to a column (2.5 × 15 cm) of DEAE-cellulose (Sigma Aldrich) pre-equilibrated with the same buffer at 4°C. Elution from the column was performed with buffer containing increasing concentrations of NaCl from 0.05 to 1 M. Approximately 3 mL fractions were collected in test tubes and the enzymatic activity of each fraction was estimated. The fraction containing enzymatic activity was purified by gel filtration after dialysis against distilled water, followed by 0.02 M sodium acetate buffer, pH 5.2 for 24 h at 4°C. The clear sample, obtained after centrifugation, was loaded onto a column (2.5 × 30 cm) of Sephadex G-75 (Sigma Aldrich) and allowed to diffuse. The eluate was collected in 2-mL fractions and monitored for enzyme activity. The active fractions were collected and dialyzed overnight against the same buffer. The homogeneity of the purified enzyme and its molecular mass were measured by SDS-PAGE.

Protein determination

Quantitative determination of protein was carried out by adding 5 mL Bradford dye reagent (0.1 g/L Sigma Coomassie Brilliant Blue G-250, 1.6 mol/L phosphoric acid, 0.8 mol/L ethanol) to 100-µL aliquots of the protein samples, incubated at room temperature for 20 min**,** and absorbance was measured at 280 nm (Bradford, 1976).

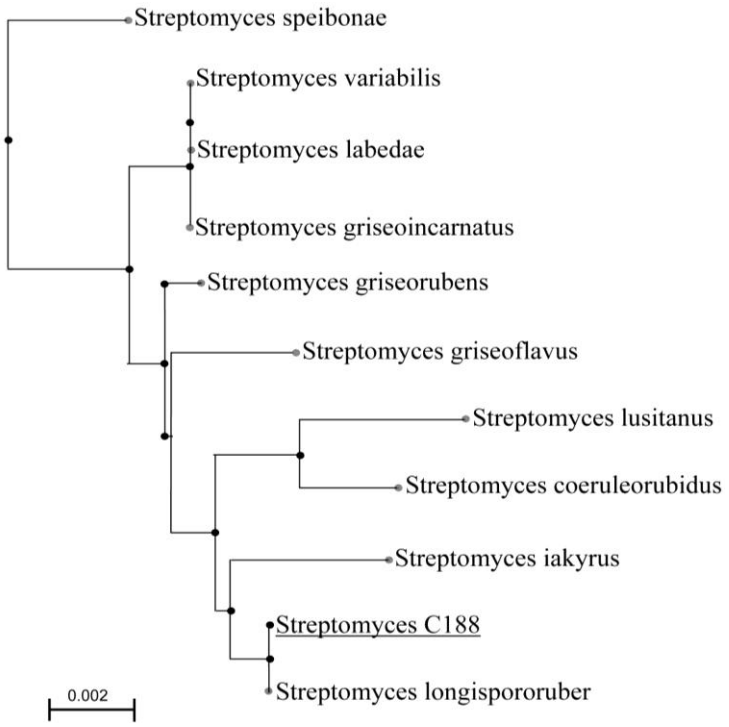

Figure 1. Neighbor-joining tree showing the phylogenetic position of *Streptomyces* C188 and related species based on partial 16S rRNA gene sequences. The scale bar indicates a 0.002 substitution per nucleotide position.

Polyacrylamide gel electrophoresis

SDS-PAGE of partially purified and pure enzyme samples was carried out as described by Sambrook et al. (1989). The proteins were stained with 0.25% Coomassie brilliant blue G-250 in aqueous solution containing 25% methanol and 5% glacial acetic acid and destained with the same solution without dye. The molecular mass of the purified enzyme was compared to standard proteins of molecular weights (MWs) between 36 and 118 kDa.

Factors affecting cellulase activity and stability

To study the effect of temperature on enzyme activity, 900 µL of 1% CMC in 20 mM phosphate buffer (pH 7) was mixed with 100 µL pure enzyme and the optimum temperature for cellulase activity was determined between 30 and 80°C. Enzyme thermostability was studied by incubating the CMC solution in 50 mM phosphate buffer (pH 7) at temperatures from 30 to 90°C for 30 min, and then residual cellulase activity was determined.

The effect of pH on enzyme activity was studied by preparing 1% CMC solution in 50 mM phosphate buffer adjusted at pH from 5 to 9, in which 900 µL samples were mixed with 100 uL of pure enzyme samples, and enzyme activity was evaluated as described. Enzyme stability at various pH levels was evaluated by preparing the pure enzyme in 50 mM phosphate buffer at pH from 5 to 9, incubated at 25°C for 30 min, and then the residual activity was determined.

Statistical analysis

Statistically significant differences between means were tested by analysis of variance and student's *t*-test by using InStat-ANOVA software. The differences between means were considered statistically significant when the test yielded a value $P < 0.05$.

RESULTS

Genetic identification of the tested *Streptomyces* isolate (C188)

The 1,426 bp sequence obtained from the test strain was aligned with all presently available 16S rRNA gene sequences in the GeneBank databases. The results show high similarity (98 to 100%) to the *Streptomyces* 16S rRNA genes. In addition, the tested nucleotide sequence of *Streptomyces* C188 shows 100% similarity to *Streptomyces longispororuber* (Accession no. NR_041147.1). The phylogenetic tree (Figure 1) revealed that *S. longispororuber is* the closest isolates in similarity to the tested strain.

Factors affecting cellulase productivity by test strain

Enzyme production by the test strain in CMC liquid medium reached 8540 U/L after 96 h incubation at 30°C. Improvement of cellulase productivity by *Streptomyces* C188 was carried out by studying the effect of organic nitrogen sources and pH on enzyme production.

Table 1. Effect of different organic nitrogen sources on growth and cellulase productivity by *S. longispororuber* in CMC broth medium at 30°C for 96 h.

Nitrogen source (concentration, %)	Cellulase productivity (U/L ± SD)	Dry cell weight (g/L)	Specific enzyme productivity (U/g)
Control	8540 ± 165	6.8	1255.9
CSL* (1%)	15490 ± 374	8.4	1844.1
CSL* (2%)	10510 ± 212	9.2	1142.4
CSL* (3%)	11480 ± 325	10.6	1083.0
Peptone (1%)	240 ± 66	4.8	50.0
Peptone (2%)	290 ± 74	5.6	51.8
Tryptone (1%)	380 ± 76	5.8	65.5
Tryptone (2%)	530 ± 87	6.7	79.1
Yeast extract (1%)	920 ± 94	7.1	129.6
Yeast extract (2%)	640 ± 62	7.3	87.7
CSL* (1%) + Peptone (1%)	6570 ± 184	8.8	746.6
CSL* (1%) + Tryptone (1%)	10320 ± 158	9.6	1075.0
CSL* (1%) + Yeast extract (1%)	8680 ± 246	9.2	943.5

CSL* = Corn steep liquor

Table 2. Effect of initial pH on growth and cellulase productivity by *S. longispororuber* in modified CMC broth at 30°C and 96 h.

pH	Cellulase productivity (U/L ± SD)	Dry cell weight (g/L)	Specific productivity (U/g)
5	1026 ± 105	4.8	213.8
5.5	11482 ± 392	6.4	1794.1
6	11482 ± 328	6.6	1739.7
6.5	15668 ± 404	7.1	2206.8
7	8551 ± 275	7.2	1187.6
7.5	6310 ± 245	7.4	852.7
8	4656 ± 212	6.8	684.7
8.5	3443 ± 196	6.2	555.3
9	2541 ± 146	5.6	453.8
9.5	1387 ± 124	5.2	266.7

Effect of organic nitrogen source

The highest level of cellulase productivity (15490 U/L) by *Streptomyces* C188 was obtained in the presence of 1% corn steep liquor (Table 1). Therefore, the modified CMC broth containing 1% corn steep liquor was used for further studies.

Effect of pH

The modified CMC broth medium was prepared with a wide range of initial pH (5-9.5). A good cellulase productivity was obtained at pH 5.5-7.5, with the highest level (15668 U/L) at initial pH 6.5 (Table 2).

Further studies were performed with 0.05 M phosphate buffer (pH 5.5-7.5) to control the pH of the fermentation medium throughout the incubation period. The greatest enzyme productivity (25084 U/L) was obtained when the fermentation pH was maintained over the incubation period at pH 6.5 (Table 3).

Purification of cellulase enzyme

The purification of cellulase enzyme is summarized in Table 4. The final preparation had 13.5% activity recovery and approximately 38.5-fold purification. The results of SDS-PAGE showed that the partially purified protein samples migrate 4 bands with relative molecular masses between 40 and 45 kDa. The final preparation of the purified

Table 3. Maintaining the pH throughout the fermentation process using 0.05 M phosphate buffer, and its effect on growth and cellulase productivity by *S. longispororuber* in modified CMC broth at 30°C for 96 h.

pH	Cellulase productivity (U/L ± SD)	Dry cell weight (g/L)	Specific productivity (U/g)
6	15628 ± 422	6.8	2298.2
6.5	25084 ± 586	7.3	3436.2
7	18197 ± 378	7.8	2332.9
7.5	11480 ± 312	7.6	1510.5

Table 4. Purification of cellulase enzyme from *S. longisporobur* culture supernatant.

Purification step	Total protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Yield (%)	Purification fold
Crude extract	32135	38420	1.2	100	1.00
Ammonium sulfate (30 - 70%)	2645	25043	9.5	65.2	7.9
DEAE-Cellulose	318	8768	27.6	22.8	23
Sephadex G-75	112	5175	46.2	13.5	38.5

cellulase enzyme had a single protein band with approximate relative molecular mass of 42 kDa (Figure 2).

Effect of temperature and pH on cellulase activity and stability

Effect of temperature

Maximum enzymatic activity was obtained at incubation temperature of 50°C. While, the thermal stability of the cellulase enzyme was up to 55°C and reduction in the activity was reached to 15% when the temperature raises up to 60°C (Figure 3).

Effect of pH

Regarding pH, maximum enzymatic activity (less than 10% reduction) was obtained at pH range 5.5 - 7, with optimum pH range at 6 - 6.5. In case of enzyme stability, enzyme preparation retained 85% or more of its activity between pH 5.5 and 7.5, while the optimum pH is 6.5 (Figure 4).

DISCUSSION

The present study focused on isolation of a high cellulase-producing *Streptomyces* isolate from soil samples of Saudi Arabia. The results of the screening by Congo red test and dinitrosalicylic acid assay revealed that the isolate coded C188 produces promising level of cellulase (8540 U/L). Genetic identification of the selected isolate was carried out by analysis of 16S rDNA gene. Based on the nucleotide sequence of the16S rDNA gene and the phylogenetic analysis, the organism is most similar to *S. longispororuber.*

Improvement of cellulase productivity by *Streptomyces* C188 was carried out by studying the effect of different nutritional factors and pH of the fermentation medium. Modification of the fermentation medium is an essential stage in the design of successful laboratory experiments, pilot scale development and manufacturing processes (Stanbury et al., 2000). Cellulase enzymes are inducible, so the presence of carboxymethyl cellulose induces enzyme production in addition to its role as carbon source. Trials were carried out to improve the cellulase productivity by adding different carbon additives (glucose, lactose, sucrose, maltose, arabinose) to the fermentation medium, but none of the tested carbon sources improved enzyme production (data not shown). This observation is well in agreement with that reported by Gautam and his collegues (2010). Accordingly, we used the same basic CMC liquid, containing carboxymethyl cellulose as carbon source, for further studies on the effect of other factors.

For studying the effect of nitrogen source, supplementation of the fermentation medium with different organic nitrogen sources was carried out. A significant increase in the enzyme productivity (15490 U/L) by the tested strain was observed in the presence of 1% corn steep liquor as compared to that of the control ($P < 0.05$). The benefit of corn steep liquor over other nitrogen sources is not only improving enzyme production but also reducing the cost of enzyme production, especially at large scales (Da Vinha et al., 2011).

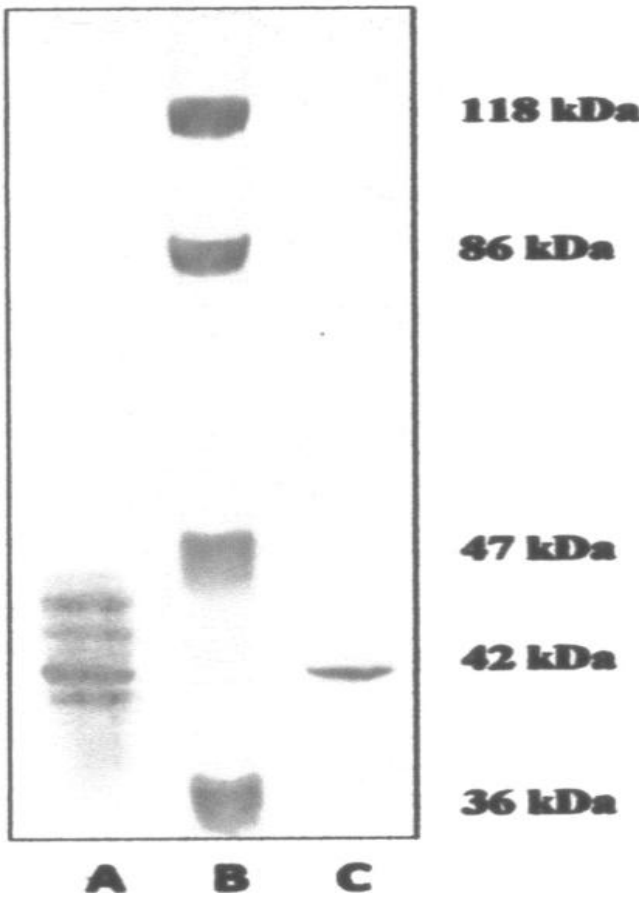

Figure 2. SDS-PAGE of (A) Partially purified protein sample, (B) molecular mass standards and (C) final preparation of the purified cellulase enzyme (β-galactosidase, 118 kDa; Bovine serum albumin, 86 kDa; Ovalbumine, 47 kDa; Carbonic anhydrase 36 kDa).

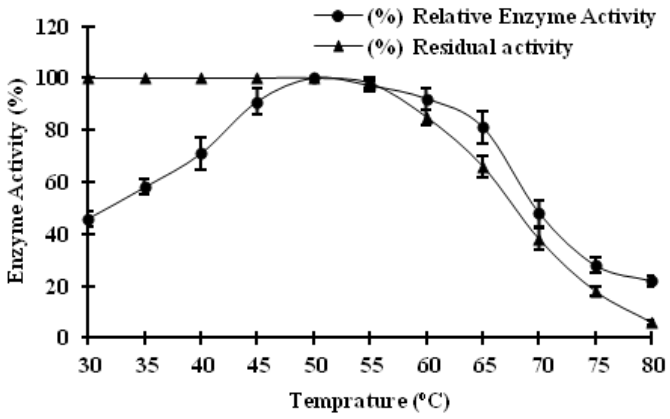

Figure 3. Effect of temperature on the enzyme activity and stability.

The pH of fermentation medium modulates microbial growth and enzyme production (Odeniyi et al., 2009). The

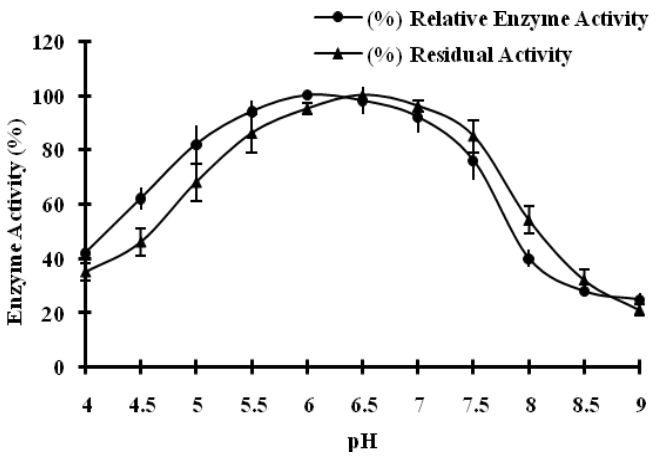

Figure 4. Effect of pH on the enzyme activity and stability.

suitable initial pH of the fermentation medium for production of cellulase enzyme by *Streptomyces* sp. was mainly located within acidic range between 5.5 and 6.5 (El-Sersy et al., 2010; Jaradat et al., 2008). However, some *Streptomyces* sp. as that isolated from East African Soda Lakes have an optimal pH of 8 (Solingen et al., 2001). In the case of *Streptomyces* C188, highest level of enzyme production was at initial pH 6.5. Regardless of the initial pH employed, the final pH of the spent media was in the alkaline range; a characteristic result in cultures of *Streptomyces* (Chen et al., 1979). In the present study, controlling the pH of the fermentation medium by 0.05 M phosphate buffer at pH 6.5 increased the enzyme productivity of *Streptomyces* C188 by 1.8 times as compared to that of the control.

Along the enzyme purification steps, a gradual increase in the specific activity to finally reach 46.2 U/mg was observed. Although the yield was only about 13.5% but over 97% of the extracted protein was removed during the purification steps and the enzyme was purified with an increase in purification fold more than 38. This decrease in yield might be due to denaturation of enzyme during the purification steps or other reasons (Begum et al., 2009).

Homogenous purified enzyme preparation was obtained as analyzed by SDS–PAGE with estimated MW to be about 42 kDa, similar to cellulase enzyme produced by *Streptomyces reticuli* 21 (Wachinger et al., 1989) and close to that produced by *Streptomyces lividans*, MW- 36 kDa (Wittmann et al., 1994). However, it was larger than 24-27 kDa of the cellulases from *Streptomyces ruber* (El-Sersy et al., 2010) and smaller than the second cellulase enzyme (MW-119 kDa) produced by *Streptomyces viridobrunneus* (Da Vinha et al., 2011).

The cellulase activity was affected by the pH and temperature of the reaction mixture. It was observed that the optimum condition of enzyme activity by *Streptomyces* C188 was pH 6-6.5 and 50°C which are in agreement with that reported by other investigators (Lima et al., 2005). However, a slightly higher optimum temperature

(55-60°C) was observed for the cellulase activity by *S. malaysiensis* (Nascimento et al., 2009) and *Streptomyces* sp. *J12* (Jaradat et al., 2008) and lower optimum temperature by *S. ruber* (El-Sersy et al., 2010).

In the case of the optimum pH, a more acidic pH (pH 4 and 4.9) was observed for the cellulase activity by *S. malaysiensis* (Nascimento et al., 2009) and *S. viridobrunneus* (Da Vinha et al., 2011). In addition, wider range of optimum pH (between 5.5 and 7.5) was reported for the cellulase activity by *S. reticuli* (Wachinger et al., 1989). The variation in the optimum pH and temperature for the cellulase activity may be due to the difference in the types of the producer strains.

In the present study, cellulase enzyme is moderately thermostable (up to 55°C). Comparatively, its optimum thermal stability was slightly higher than that produced by *S. viridobrunneus* (Da Vinha et al., 2011) and lower than that produced by *Mucor circinelloides* (Saha et al., 2004). Regarding the effect of pH on the enzyme stability, the optimum pH in the present study (6.5) was similar to that produced by *Bacillus sublitis* YJ1 (Yin et al., 2010).

Thus, a high-cellulase producing *Streptomyces* isolate, identified as *S. longispororuber*, was isolated from Saudi Arabia, and its enzyme productivity was improved by modifying the nutritional conditions and pH of the fermentation medium. The produced cellulase enzyme has MW of 42 kDa and its maximum enzymatic activity was obtained at 50°C and pH 6.5, while the maximum stability was at 55°C and pH 6-6.5.

ACKNOWLEDGEMENT

This paper was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (3-166-D1432). The authors, therefore, acknowledge with thanks DSR technical and financial support.

REFERENCES

Barros RRO, Oliveira RA, Gottschalk LMF, Bon EPS (2010). Production of cellulolytic enzymes by fungi *Acrophialophora nainiana* and *Ceratocystis paradoxa* using different carbon sources. Appl. Biochem. Biotechnol. 161:448-454.

Begum F, Absar N, Alam MS (2009). Purification and characterization of extracellular cellulase from *A. oryzae* ITCC-4857.01. J. Appl. Sci. Res. 5:1645-1651.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-5.

Chen WP, Anderson AW, Han YW (1979). Production of glucose isomerase by Streptomyces flavogriseus. Appl. Environ. Microbiol. 37:324-331.

Da Vinha FNM, Gravina-Oliveira MP, Franco MN, Macrae A, Da Silva Bon EP, Nascimento RP, Coelho RRR (2011). Cellulase production by *Streptomyces viridobrunneus* SCPE-09 using lignocellulosic biomass as inducer substrate. Appl. Biochem. Biotechnol. 164:256-267.

Edwards U, Rogall T, Bocker H, Emade M, Bottger E (1989). Isolation and direct complete nucleotide determination of entire genes. characterization of a gene coding for 16s ribosomal DNA. Nucleic Acid Res. 17:7843-7853.

El-Sersy NA, Abd-Elnaby H, Abou-Elela GM, Ibrahim HAH, Toukhy NM (2010). Optimization, economization and characterization of cellulase produced by marine *Streptomyces ruber.* Afr. J. Biotechnol. 9:6355-6364.

Gautam SP, Bundela PS, Pandey AK, Jamaluddin M, Awasthi MK, Sarsaiya S (2010). Cellulase production by *Pseudomonas sp*. isolated from municipal solid waste compost. Int. J. Acad. Res. 2:330-333.

Jaradat Z, Dawagreh A, Ababneh Q, Saadoun I (2008). Influence of culture conditions on cellulase production by *Streptomyces* sp. (Strain J2). Jordan J. Biol. Sci. 1:141-146.

Kirk O, Borchert TV, Fuglsang CC (2002). Industrial enzyme applications. Curr. Opin. Biotechnol. 13:345-351.

Lima AO, Quecine MC, Fungaro MH, Andreote FD, Maccheroni W, Jr, Araújo WL, Silva-Filho MC, Pizzirani-Kleiner AA, Azevedo JL (2005). Molecular characterization of a beta-1,4-endoglucanase from an endophytic *Bacillus pumilus* strain. Appl. Microbiol. Biotechnol. 68:57-65.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426-428.

Nascimento RP, Junior NA, Pereira N Jr, Bon EP, Coelho RR (2009). Brewer's spent grain and corn steep liquor as substrates for cellulolytic enzymes production by *Streptomyces malaysiensis*. Lett. Appl. Microbiol. 48:529-35.

Odeniyi OA, Onilude AA, Ayodele MA (2009). Production characteristics and properties of cellulase/polygalacturonase by a *Bacillus coagulans* strain from a fermenting palm-fruit industrial residue. Afr. J. Microbiol. Res. 3:407-417.

Ponnambalam AS, Deepthi RS, Ghosh AR (2011). Qualitative display and measurement of enzyme activity of isolated cellulolytic bacteria. Biotechnol. Bioinf. Bioeng. 1:33-37.

Pospiech A, Neumann B (1995). A versatile quick-prep of genomic DNA from gram-positive bacteria. Trends Genet. 11:217-8.

Saha BC (2004). Production, purification and properties of endoglucanase from a newly isolated strain of *Mucor circinelloides*. Process Biochemistry 39:1871-1876.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning- a Laboratory Manual. Cold Spring Harber Laboratory, Cold Spring Harber, N.Y.

Sanger FS, Nicklen S, Coulson AR (1977). DNA sequencing with chain terminator inhibitors. Proc. Natl. Acad. Sci. 74:5463-5467.

Solingen VP, Meijer D, Kleij WA, Branett C (2001). Cloning and expression of an endocellulase gene from a novel streptomycet isolated from an East African soda lake. *Extremophiles* 5:333-341.

Stanbury PF, Whitaker A, Hall SJ (2000). Principles of Fermentation Technology. Butterworth-Heinmann, Heinmann, Oxford.

Tahtamouni MEW, Hameed KM, Saadoun IM (2006). Biological control of *Sclerotinia sclerotiorum* using indigenous chitinolytic actionmycetes in Jordan. Plant Pathol. J. 22:107-114.

Wachinger G, Bronnrnmeier K, Walter WL, Schrempf H (1989). Identification of mycelium-associated cellulase from *Streptomyces reticuli*. Appl. Environ. Microbiol. 55:2653-2657.

Wittmann S, Sharech F, Kluepeel D, Morosoli R (1994). Purification and characterization of the CelB endoglucanase from *Streptomyces lividans* 66 and DNA sequence of the encoding gene. Appl. Environ. Microbiol. 60:1701-1703.

Yin L, Lin H, Xiao Z (2010). Purification and characterization of a cellulase from *Bacillus subtilis* AJ1. J. Marine Sci. and Technol. 18:466-471.