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Full Length Research Paper

Optimization, economization and characterization of cellulase produced by marine *Streptomyces ruber*

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Cellulase is a very important enzyme due to its great industrial applications. Six marine strains of actinomycetes were screened for their carboxymethyl cellulase (CMCase) productivity. *Streptomyces ruber* was chosen to be the best producing strain. The highest enzyme production (25.6 U/ml) was detected at pH 6 and 40°C after 7 days of incubation. Plackett-Burman design was applied to optimize the different culture conditions affecting enzyme production. Results showed that a high concentration of KH_2PO_4, and a low concentration of $MgSO_4$ had a significant effect on enzyme production. Rice straw was used as a low cost source of cellulose. It was found that 30 g/l rice straw was the suitable concentration for maximum enzyme production. Partial purification of cellulase enzyme using an anion-exchange chromatography resulted in the detection of two different types of CMCases, type I and II, with specific activity of 4239.697 and 846.752 U/mg, respectively. Moreover, estimation of their molecular weight revealed 27.0 kDa for cellulase type I and 24.0 kDa for cellulase type II. It could be concluded that *S. ruber* is a powerful cellulase producer strain under our tested experimental conditions.

Key words: Cellulase production, *Streptomyces ruber,* Plackett-Burman design, rice straw, enzyme characterization.

INTRODUCTION

Cellulases have attracted much interest because of the diversity of their applications. The major industrial applications of cellulases are in textile industry for 'biopolishing' of fabrics and for producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness. Moreover, they are used in animal feeds for improving nutritional quality and digestibility, in processing of fruit juices, and in baking, while de-inking of paper is yet another emerging application. A potential challenging area where cellulases would have a central role is the bioconversion of renewable cellulosic biomass to commodity chemicals (Ibrahim and El-Diwany, 2007).

Actinomycetes, one of the known cellulase producers, has attracted considerable research interest due to its potential application in the recovery of fermentable sugars from cellulose that can be of benefit to human consumption and to the ease of their growth (Jang and Chen, 2003; Arunachalam et al., 2010). Streptomycetes are the largest and well-studied group of actinomycetes. A wide variety of bacteria are known for their production of hydrolytic enzymes with streptomycetes being the best known enzyme producers (Chellapandi and Jani, 2008).

The optimization of fermentation conditions is an important problem in the development of economically feasible bioprocesses. Combinatorial interactions of medium

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Abbreviations: CMC, Carboxymethyl cellulose; **CMCase,** carboxymethyl-cellulase; **DNS,** dinitrosalicylic acid; **DEAE,** diethylaminoethyl.

components with the production of the desired compounds are numerous and optimum processes may be developed using an effective experimental design procedures (Hao et al., 2006; El-Sersy et al., 2010).

Rice cultivation produces large quantities of straw, as an agricultural waste, ranging from 2 to about 9 tons/ha globally. Components of rice straw are mainly cellulose and hemicellulose encrusted by lignin, in addition to a small amount of protein, which makes it high in C:N ratio (Abdulla, 2007). Products of agricultural practices result in the generation of numerous structural plant components which contribute to environmental pollution problems. One of the easiest ways of handling pollution problems is by the re-use of these agricultural wastes in the production of highly important useful enzymes (Odeniyi et al., 2009a).

In the present study, we aim to study the optimization and economization of cellulase production using marine *Streptomyces ruber* from a low-cost source. Moreover the study extended to investigate the characterization of the enzyme.

MATERIALS AND METHODS

Microorganisms

Six actinomycetes isolates used in the current investigation were kindly provided by Dr. Gehan Abou Elela (Associate professor of Marine Microbiology, National Institute of Oceanography and Fisheries, Alexandria - Egypt). Isolates were recovered from marine sediments in a previous study, They were tentatively identified as *Streptoverticillium morookaense, Streptomyces globosus, Streptomyces alanosinicus, S. ruber, Streptomyces gancidicus and Nocardiopsis aegyptia,* following Bergy's Manual of Systematic Bacteriology (Holt and Williams, 1994) by Al-Azhar University Fermentation Biotechnology and Applied Microbiology (Ferm. BAM) Center, Egypt.

Electron microscopy studies

Electron microscopy was performed using the cover slip technique. The cover slip was cut with a glass file and a suitable fragment with growth on it was chosen. It was mounted on a specimen-tube, coated with gold-palladium under vacuum and examined with a scanning electron microscope (Joel ISM-5300) operating at 10 KV.

Screening for cellulases producing actinomycetes strains

A preliminary analysis for cellulolytic activity was conducted using Congo red dye. All strains were grown on carboxymethyl cellulose (CMC) agar containing (g/l) KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5; $FeSO_4.7H_2O$, 0.01; $MnSO_4.H_2O$, 0.01; NH_4NO_3, 0.3; (CMC), 10.0 and agar 12.0. The pH was adjusted to 7.0 with 1.0 M NaOH. The CMC agar plates were incubated at 30°C for 7 days to allow for the secretion of cellulase. At the end of the incubation, the agar medium was flooded with an aqueous solution of Congo red (1% w/v) for 15 min. The Congo red solution was then poured off, and the plates were further treated by flooding with 1.0 M NaCl for 15 min. The formation of a clear zone of hydrolysis indicated cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select the highest cellulase activity producer. The largest ratio was assumed to contain the highest activity (Ariffin et al., 2006).

Production of crude cellulases

The production of crude enzyme was carried out in the same medium used for screening without the addition of agar. A loop full of culture from agar plates was inoculated into glass tubes containing 5 ml of production medium, and incubated at 120 rpm and 30°C. This culture was then inoculated ($9x10^4$ CFU/ml) into a 250-ml capacity Erlenmeyer flask containing 100 ml of the same medium, 2 ml aliquots were withdrawn and were centrifuged at 10,000 g for 10 min. Cellulase activities were measured in cell-free supernatant which was used as the source of crude cellulase enzymes.

Carboxymethyl-cellulase (CMCase) activity

CMCase activity was assayed using a modified method described by Wood and Bhat (1998) with some modifications. 0.2 ml of culture filtrate was added to 1.8 ml of 1% CMC prepared in 0.05M sodium citrate buffer (pH 4.8) in a test tube and incubated at 40°C for 30 min. The reaction was terminated by adding 3.0 ml of dinitrosalicylic acid (DNS) reagent and by subsequently placing the reagent tubes in a water bath at 100°C for 15 min. 1 ml of Rochelle salt solution (40 g Rochelle salt in distilled water to make the volume 100 ml) was then added to stabilize the colour. Using spectrophotometer, absorbance was recorded at 575 nm against the blank (0.05 M sodium citrate buffer). One unit of CMCase activity was expressed as 1.0 µM of glucose liberated per ml enzyme per min (Ariffin et al., 2006). By using a calibration curve for glucose, one unit of enzyme activity was defined as the amount of enzyme that released 1 µM of glucose per min.

Reducing sugars content

Reducing sugars analysis was conducted by using 2 ml of sample which was added to 3 ml of DNS and boiled for 15 min. After boiling, 1 ml of Rochelle salt was added. The absorbance was recorded at 575 nm using a spectrophotometer against the blank of distilled water (Ariffin et al., 2006).

Protein determination

Protein content was determined by the assay method which is based on the method of Bradford (1976). In this assay, 5 ml dye reagent was pipetted into 100 µl of sample solution. The mixture was then incubated at room temperature for at least 5 min, but not more than 60 min. The absorbance was measured at 280 nm against the blank of deionized water.

Biomass yield

Culture broth was centrifuged and the cell pellet was washed twice with phosphate buffer (0.1 M, pH 7). The cell mass was dried at 80°C to constant weight (Bidlan et al., 2007).

Effect of temperature and pH on cellulase production

The influence of incubating temperature on cellulase production was

Table 1. Screening test of different actinomycetes strains grown on carboxymethyl cellulose (CMC) and their clearance zones (mm) after 7 days of incubation.

Strain	Diameter of the colony (mm)	Diameter of clearance zone (mm)
Streptoverticillium morookaense	6	10
Streptomyces globosus	5	10
Streptomyces alanosinicus	7	15
Streptomyces ruber	9	25
Streptomyces gancidicus	7	15
Nocardiopsis aegyptia	5	10

determined by measuring enzyme activity at temperatures ranging from 30 to 60°C under standard assay conditions. The effect of pH-value on enzyme production was determined by measuring the enzyme activity at different pH-values ranging from 5 to 9.

Experimental design and optimization

Plackett-Burman is a technique devoted to the screening of controlled experimental factors and the measurement of their responses, according to one or more selected criteria. A prior knowledge and understanding of the process and the process variables under investigation are necessary for achieving more realistic results. Plackett-Burman design was used to pick factors that influence cellulase production significantly and insignificant ones were eliminated in order to obtain smaller, more manageable set of factors (Hao et al., 2006).

For each variable, a high (+) and low (-) levels were tested. The examined variables in this experiment and their levels are shown in Table 2. Eight different trials were performed in duplicates. Rows in Table 3 represent the different trials (row number 9 represents the basal control). The main effect of each variable was determined with the following equation:

$$Ex_i = (Mi^+ - Mi^-) / N$$

Where, Ex_i is the variable main effect, and Mi^+, Mi^- are the enzyme produce (units) in the trials, where the independent variable was present in high and low concentrations, respectively, and N is the number of trials divided by two. Statistical t-values for equal unpaired samples were calculated using Microsoft Excel to determine the variable significance. From main effect results, an optimized medium was predicted which will give maximum enzyme productivity.

Verification experiment

A verification experiment was carried out in duplicates. The predicted optimum levels of the independent variables were examined and compared to the basal condition setting and the average of enzyme production was calculated.

Cellulase production using agro-industrial residues

Rice straw was provided from a rice field at Kafr El-Dawar City. It was dried at 50°C then milled well several times until it gave a powdery appearance. Optimized medium was prepared without carbon and nitrogen sources but supplemented with different concentrations (10, 15, 20, 25, 30 and 40 g) of rice straw. All flasks were incubated and enzyme activity was assayed.

Partial purification of cellulase enzyme and polyacrylamide gel electrophoresis (PAGE)

Partial purification of cellulase enzyme produced from the growth of *S. ruber* on rice straw under optimized conditions, was performed using KTA Fast Protein Liquid Chromatography (FPLC) (Amersham Pharmacia Biotec. The diethylaminoethyl cellulose (DEAE)-sepharose CL 6B column as an anion-exchange liquid chromatography technique was used for separation and purification of cellulase enzyme, while the running buffer was 20 mM sodium acetate (pH 6) and the elution buffer was 1.0 M NaCl in 20 mM sodium acetate (pH 6). The elution rate was 1.0 ml/min in a sample volume of 5 ml.

The purified cellulase was desalted and concentrated using the ultrafiltration tubes at a speed of 3,000 rpm for 30 min at 4°C in Centricon 10 (Amicon, USA) ultrafiltration concentrators (membrane cut off of 10 kDa).

For the detection of the molecular weight and cellulase enzyme homogeneity, the denaturing sodium dodecylsulphate-PAGE was used (Laemmli, 1970), on SCIE-PLAS TV100 YK-EBSYS TV-Modular Electroblotting System with 10% polyacrylamide. Fermentas protein was used as a molecular weight marker.

RESULTS

Screening of cellulase producers

Screening of actinomycetes was conducted using the Congo red test as a preliminary study for choosing the best cellulase producers. After 7 days of incubation, all strains of actinomycetes showed signs of growth on CMC agar and demonstrated positive results in the Congo red test. Table 1 shows that *S. ruber* gave the highest ratio of clear zone diameter to colony diameter. This indicated more cellulose degradation in CMC agar plates cultured with *S. ruber* as compared to plates cultured with other strains. Growth of *S. ruber* after 7 days at 30°C was illustrated by scanning electron micrograph (Figure 1).

Effect of temperature and pH-value on enzyme production

Using *S. ruber* as a cellulase producer, the effect of temperature on the production of crude cellulases was determined at various temperatures ranging from 30 to

Table 2. Independent variables affecting cellulase production and their levels in the Plackett-Burman design.

Factor (g/l)	Symbol	Level		
		-1	0	1
KH_2PO_4	KH_2	0.5	1	1.5
$MgSO_4$	Mg	0.1	0.5	1.5
NaCl	Na	0.0	0.5	1.5
$FeSO_4$	Fe	0.0	0.01	0.05
$MnSO_4$	Mn	0.0	0.01	0.05
NH_4NO_3	NH	0.1	0.3	0.5
Inoculum size (ml)	IS	0.5	1	1.5

Table 3. The applied Plackett-Burman experimental design for seven cultural variables and their enzyme activity results.

Trials	KH_2	Mg	Na	Fe	Mn	NH	IS	Enzyme activity (U/ml)	Biomass (mg/ml)	Reducing sugar (µg/ml)	Protein content (mg/ml)	Specific activity (U/mg)
1	-1	-1	-1	1	1	1	-1	27.7	5.79	257.14	0.39	71.03
2	1	-1	-1	-1	-1	1	1	35.6	6.1	300	0.52	68.46
3	-1	1	-1	-1	1	-1	1	17.9	0.697	120	0.22	81.36
4	1	1	-1	1	-1	-1	-1	19.4	3.61	197.1	0.25	77.6
5	-1	-1	1	1	-1	-1	1	18.4	0.92	177.1	0.23	80
6	1	-1	1	-1	1	-1	-1	39.5	11.12	385	0.62	63.71
7	-1	1	1	-1	-1	1	-1	18.3	3.32	187.1	0.21	87.14
8	1	1	1	1	1	1	1	24.7	3.65	202.8	0.33	74.85
9	0	0	0	0	0	0	0	25.6	5.31	218	0.32	80

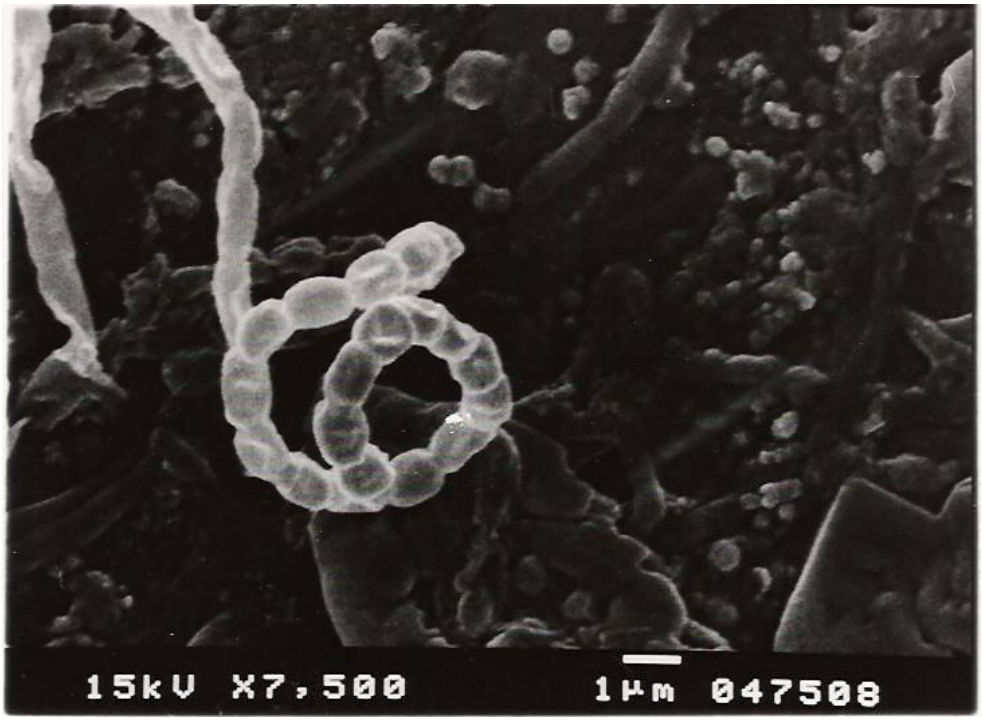

Figure 1. Scanning electron micrograph showing the growth of *S. ruber* after 7 days at 30°C.

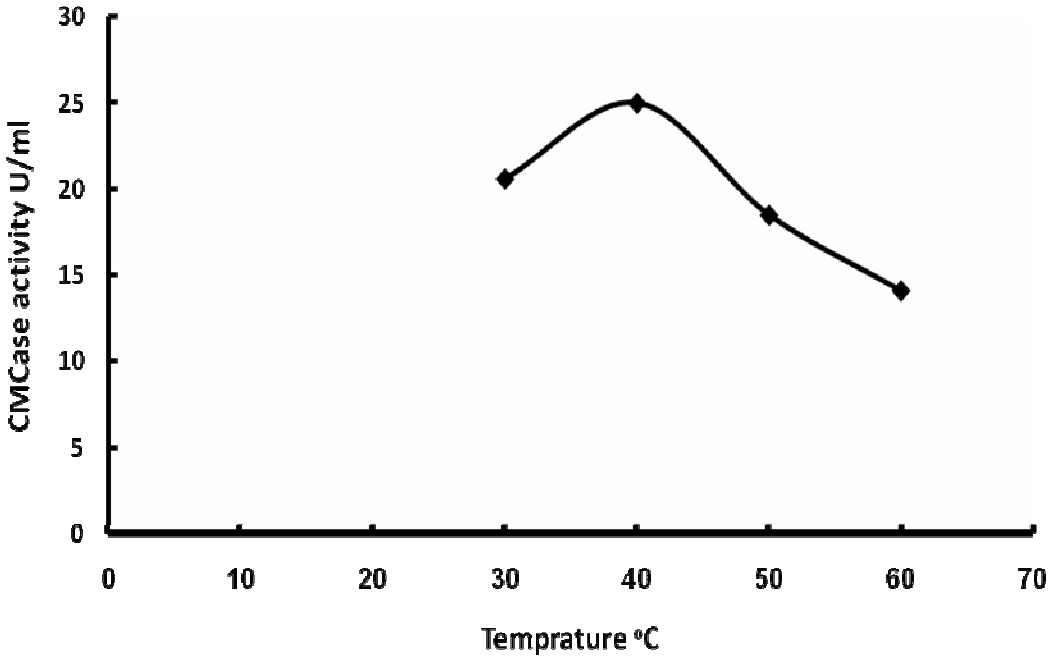

Figure 2. Effect of temperature on the cellulase enzyme activity produced from *S. ruber*.

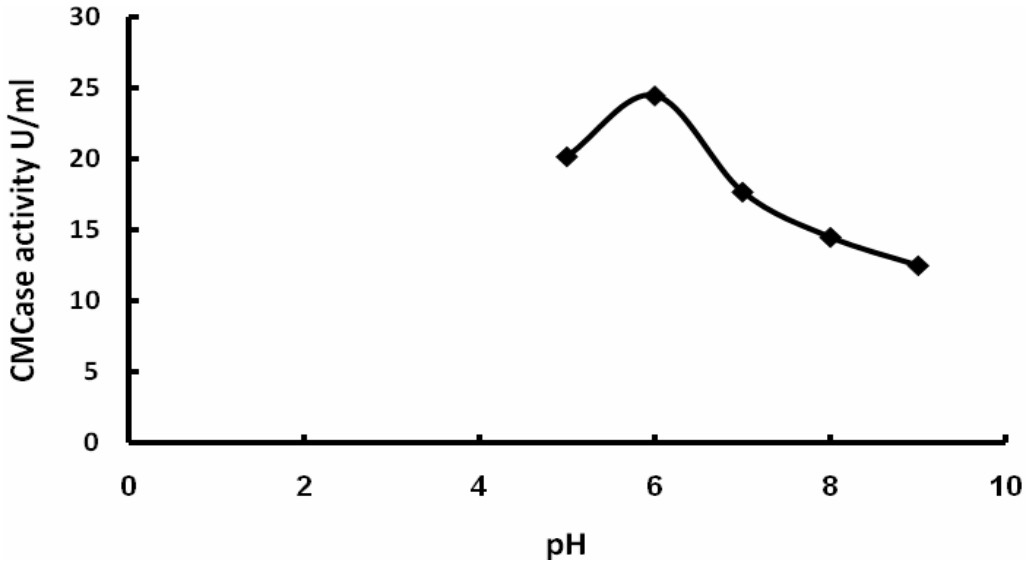

Figure 3. Effect of pH-value on the cellulase enzyme activity produced from *S. ruber*.

60°C at pH 7 (Figure 2). The enzyme showed a good production between 35 to 45°C with maximum activity at 40°C. The effect of pH-value on cellulase enzyme activity produced from *S. ruber* was examined at various pH values ranging from 5.0 to 9.0 as shown in Figure 3. The enzyme shows high activity at a broad range of pH values (pH 5.5 - 7) with optimal pH at 6.0. The enzyme production had about 50% decrease at pH 9.

Evaluation of different parameters affecting cellulase production

Plackett-Burman design has been employed to evaluate the significant effect of the seven different culture elements on the production of cellulase using a basal medium. The main effect of each constituent on the cellulase production (Table 4) was calculated as the difference between

Table 4. Statistical analysis for the results applied to the Plackett-Burman experimental design.

Variable	Enzyme activity (U/ml)		Biomass (mg/ml)	
	Main effect	*t*-value*	Main effect	*t*-value
KH_2PO_4	9.22	1.8	3.44	-4.13
$MgSO_4$ (g/l)	-10.2	-2.1	-3.18	-3.99
NaCl ((g/l)	0.05	0.01	0.7	-3.47
$FeSO_4$ ((g/l)	-5.2	-0.9	-1.81	-3.6
$MnSO_4$	4.6	0.7	1.82	-3.61
NH_4NO_3	2.8	0.4	0.635	-3.47
Inoculum size (ml)	-2.1	-0.3	-3.12	-3.98

*t-value significant at the 1% level = 3.70; 5% level = 2.45; 10% level = 1.94; 20% level = 1.37.
Standard t-values were obtained from statistical methods (Cochran and Snedecor, 1989).

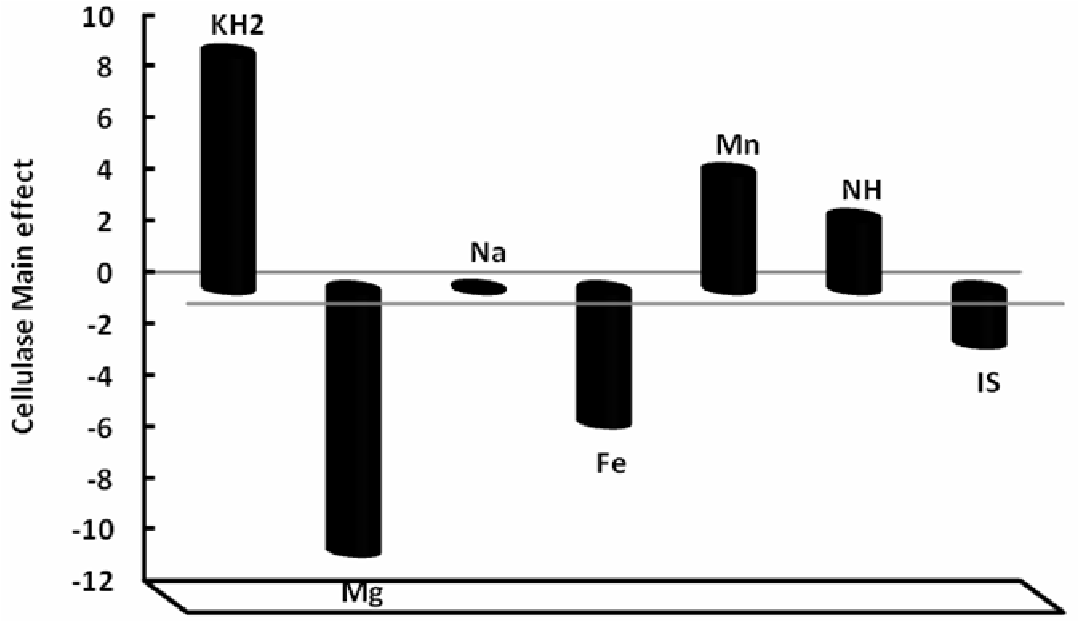

Figure 4. Enzyme production main effect of the medium constituents after applying Plackett-Burman experimental design.

the average response measurement calculated at the higher (+) and lower (-) levels of the constituent (Table 3). Main effect results (Figure 4) showed that KH_2PO_4 had a highly positive main effect which positively affected the enzyme produce. Also, $MnSO_4$ and NH_4NO_3 had positive main effect. On the other hand, $MgSO_4$, $FeSO_4$ and inoculum size showed considerable high negative main effect on enzyme production. Moreover, main effect results were confirmed by calculating t-test. KH_2PO_4, and $MgSO_4$ showed significant effect on enzyme production (Table 4). Figure 5 illustrates the interaction between KH_2PO_4, and $MgSO_4$ concentrations (g/l) on the cellulase production. We can notice from this figure that the high concentration of KH_2PO_4 with the low concentration of $MgSO_4$ increased the cellulase production. Finally, it can be concluded that the optimum formula of cellulase production medium is as follows (g/l): KH_2PO_4, 1.5; $MgSO_4$, 0.1; $MnSO_4$, 0.05; NH_4NO_3, 0.5; NaCl, 1.5 with inoculum size of 0.5 ml.

Verification experiment

A duplicate of experiment was performed to verify the optimization result in order to validate the developed optimized medium. The optimized medium recorded a higher enzymatic activity (40 U/ml) than that of the basal by 1.6 fold increase. These results confirm the validity of

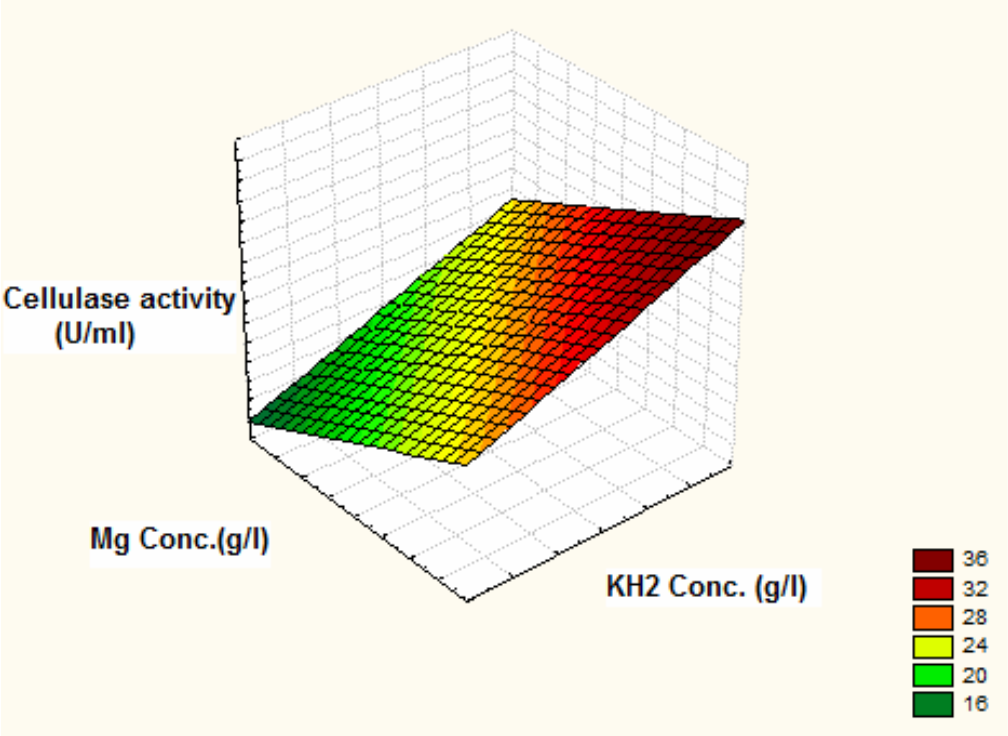

Figure 5. Interaction between KH_2PO_4, and $MgSO_4$ concentrations (g/l) on the activity of the cellulase enzyme (U/ml).

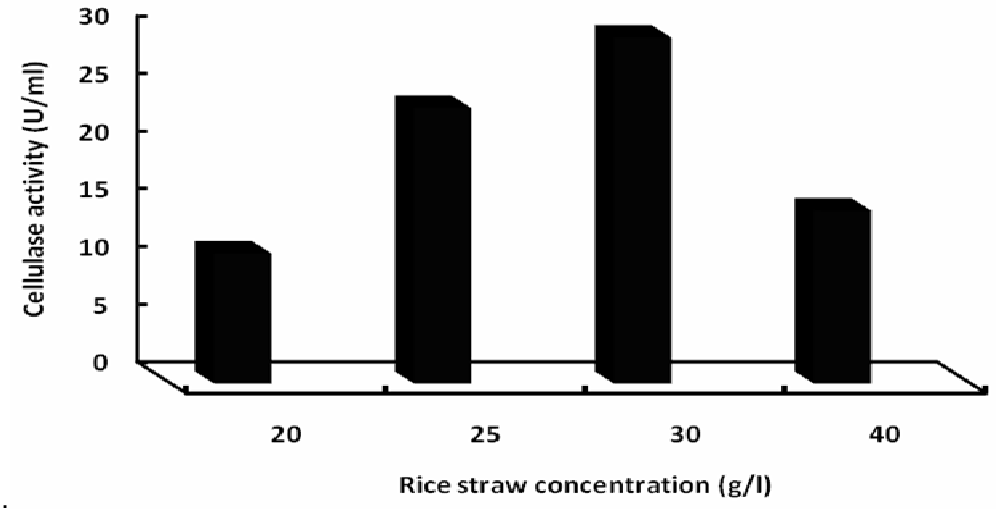

Figure 6. Effect of using rice straw at different concentrations on cellulase enzyme activities.

the optimized medium.

Effect of using rice straw at different concentrations on cellulase enzyme activity

This experiment was done for the determination of the optimum rice straw concentration which can be used for giving rise to a maximum cellulase production. Different concentrations of rice straw were used as illustrated in Figure 6. Results revealed that a concentration of 30 g/l rice straw could give a high cellulase enzyme activity (30 U/ml) while increasing rice straw concentration to 40 g/l, dramatically decreases the enzyme activity (15 U/ml) to

Table 5. Results of partially purified cellulase enzyme.

Cellulase types	Protein content (mg/ml)	Cellulase activity (U/ml)	Specific activity (U/mg)
Total Cellulase in the culture filtrate	1.55	659.25	425.32
Cellulase type I	0.099	419.730	4239.697
Cellulase type II	0.342	289.415	846.752

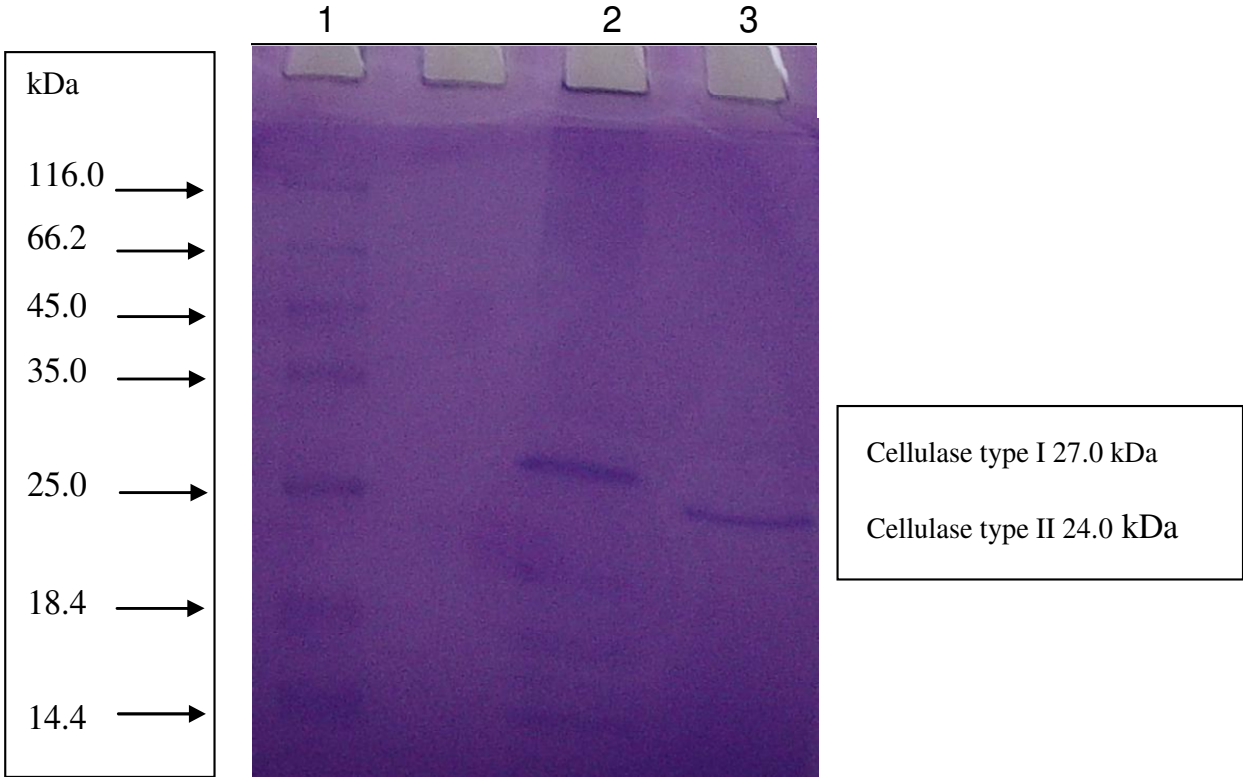

Figure 7. SDS-gel polyacrylamide electrophoresis (SDS-PAGE) showing the partially purified CMCases. Lane 1: Fermentas protein standard (cat. No. SM0431) with range from 14.4 - 116.0 kDa; lane 2: partially purified cellulase enzyme type I; lane 3: partially purified cellulase enzyme type II.

50%.

Partial purification of cellulase and polyacrylamide gel electrophoresis

The enzyme was partially purified using the DEAE-sepharose CL 6B as an anion-exchange chromatography and the fractions were tested for the CMCases. This resulted in the appearance of two different type of CMCases, type I and type II. The corresponding fractions of each type was pooled, dialyzed and tested for protein contents and cellulase activity (Table 5).

Comparing their specific activities with the specific activity of the culture filtrate indicated that the anion-exchange chromatography as a separation technique was an excellent method for cellulase purification, since the purification-fold for cellulase type I was found to be almost 10 times while purification-fold for cellulase type II was found to be almost two times.

SDS-PAGE with Coomassie-brilliant blue staining was conducted to determine the molecular weight of the two types of cellulases. The estimated molecular weight of cellulase type I was 27.0 kDa, while for cellulase type II, it was 24.0 kDa (Figure 7).

DISCUSSION

Streptomyces species have always been a source of thousands of bioactive compounds. Enzymes are one of the important products of this unusual group of bacteria. *Streptomycetes* sp. with potential cellulolytic activity is subjected to produce endoglucanase in liquid culture

(Chellapandi and Jani, 2008). Most of the *Streptomyces* isolates recovered from the different soils of Jordan produced fiber hydrolytic enzymes. Cellulase, which is considered one of the most important hydrolytic enzymes, was produced by most of the isolates (94%) (Jaradat et al., 2008; Arunachalam et al., 2010) studied in cellulose producing actinomycetes from soil of Southern-West Ghats, Tamilandu, India. Screening of actinomycetes strains was conducted by using the Congo red test. Since the sole carbon source in CMC agar was cellulose, the result of the test was strong evidence that cellulase was produced.

S. ruber gave the highest ratio of clear zone diameter to colony diameter. This indicated more cellulose degradation in CMC agar plate cultured with *S. ruber* as compared to plates cultured with the other strains.

Temperature and pH-values were found to be important parameters that influenced enzyme activities and production (Odeniyi et al., 2009b). CMCase enzyme from *S. ruber* was found active over a pH range of 5.5 - 7 with maximum activity at pH 6. Theberge et al. (1992) showed that the optimum pH for endoglucanase from a strain of *Streptomyces lividans* was 5.5. Jaradat et al. (2008) also found that CMCase enzyme from *Streptomyces sp*. (strain J2) was active over a pH range of 4 - 7 with maximum activity at pH 6 . Ariffin et al. (2006) reported that maximum activity of CMCase from *Bacillus pumilus* was detected at pH 6. However, Solingen et al. (2001) studied the alkaline novel *Streptomyces* species isolated from east African soda lakes which showed an optimal pH of 8.

The maximum CMCase activity of *S. rubber* was recorded at 40°C and the optimum range, 35 - 45°C. These results are similar to that reported by Alam et al. (2004) who studied the heavy growth and high cellulase activity by *Streptomyces omiyaensis* at 35 and 40°C. Furthermore, McCarthy (1987) reported an optimal temperature for cellulase activity in the range of 40 - 55°C for several *Streptomyces* species including *Streptomyces lividans, Streptomyces flavogrisus*, and *Streptomyces nitrosporus*. On the other hand, higher temperatures were optimum for CMCase production. Jaradat et al. (2008) found that the maximum CMCase activity of *Streptomyces sp.* (isolate J2) was recorded at 60°C with no significant difference ($p < 0.05$) between 50 and 60°C. In addition, Jang and Chen (2003) described a CMCase produced by a *Streptomyces* T3-1 with an optimum temperature of 50°C whereas Schrempf and Walter (1995) described a CMCase production by a *Streptomyces reticuli* at an optimum temperature of 55°C.

Prolonged incubation periods (7 days) were required to obtain maximum enzymatic production by streptomycetes and that agrees with Alam et al. (2004) and Arunachalam et al. (2010).

Statistical experimental designs are powerful tools for the rapid search of key factors from a multivariable system and minimizing the error in determining the effect of parameters and the results are achieved in an economical manner (El-Sersy, 2007; Abou-Elela et al., 2009). One of the advantages of the Plackett-Burman design is that it helps to rank the effect of different variables on the measured response independent of its nature (either nutritional or physical factor) or sign (whether contributes positively or negatively) (Youssef and Berekaa, 2009).

Youssef and Berekaa (2009) studied the production of endoglucanase by *Aspergillus terreus* by applying the Plackett-Burman design for optimization of process parameters and this study agreed with our results in that, both KH_2SO_4 and $MgSO_4$ positively affected CMCase production. High levels of KH_2SO_4 and low levels of $MgSO_4$, maximize enzyme production. Hao et al. (2006) studied optimization of the medium for the production of cellulase by the mutant *Trichoderma reesei* WX-112 using response surface methodology and the results showed that the presence of K_2HPO_4 positively affected cellulase production. In our study, the predicted medium for optimum production is as follows: KH_2SO_4, 1.5; NaCl, 1.5; $MnSO_4$, 0.05; NH_4NO_3, 0.5; $MgSO_4$, 0.1 and inoculum size, 0.5 ml. Moreover, verification experiment using this medium, increased cellulase activity by 1.6 folds.

Rice cultivation produces large quantities of straw as an agriculture waste. Components of rice straw are mainly cellulose and hemicellulose encrusted by lignin, in addition to a small amount of protein. It is resistant to microbial decomposition compared to straw from other protein-rich grains such as wheat and barley (Parr et al., 1992). Egypt is the largest rice producer in the Near East region (Sabaa and Sharaf, 2000). Currently, the major practice to eliminate such massive amounts of post-harvest rice residues is field open air burning. The produced black smoke represents a threat to public health; it also introduces carbon monoxide and some nitrogen dioxide, which has statistically significant effect on asthma morbidity (Schwartz et al., 1993). Low cost production of cellulases from different wastes had been studied by many workers. Shabeb et al. (2010) studied the low cost production of cellulase from molasses by *B. subtilis* KO and the economic value of the product. Immanuel et al. (2007) reported that *Aspergillus niger* and *Aspergillus fumigatus* were capable of producing cellulase enzyme optimally at 40 and 50°C during growth on coir waste and sawdust, respectively. In this study, microbial decomposition by exhausting rice straw (as pollutant) and the economic production of cellulase were valuable aims to environmental protection and industrial progress.

Partial purification of cellulase was conducted by Bajaj et al. (2009) where the endoglucanase was purified to the extent of 9.06 folds by salt precipitation and DEAE-cellulose chromatography. Moreover, the study was extended to investigate endoglucanase molecular weight which was approximately 54 kDa as examined by SDS-PAGE. In our study, partial purification of cellulase using

anion-exchange chromatography resulted in two types of cellulases, type I and II. Cellulase type I was purified to the extent of 10 fold while type II was about 2 fold. Molecular weight estimation using SDS-PAGE showed that the estimated molecular weight of cellulase type I was 27.0 kDa while for cellulase type II, it was 24.0 kDa. Conclusively, to the best of our knowledge, this is the first report on the high level of production of cellulase by *S. ruber*, from rice straw.

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