

## An Investigation for Cellulase Activity of a Novel Antibiotic producing *Streptomyces* sp. Isolate H-1 from Egyptian Mangrove Sediment

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**Abstract:** The aim of the current study was to investigate the cellulolytic activity of a previously isolated and identified isolate from mangrove sediment from Hurgada natural protectorates in Egypt as mention below. The Actinomycete isolate, *Streptomyces* sp.H-1, was known to produce aromatic antibiotic, possess broad spectrum of antibacterial activity, very similar to the antibiotic Tubermycin- -A. The results of the current study show that this isolate also has a very powerful cellulase enzyme system capable of degrading natural cellulosic biomass residues, such as crystalline cellulose derived from cotton seed linters, both *In-vivo* and *In-vitro*. In the meanwhile, the antibiotic production also was reserved. Study of enzyme production optimization revealed that its best production was under shaking incubation (200 rpm) for seven days at pH 7, 35 °C, and crystalline cellulose concentration 2 % (w/v). In each case, it recorded the highest enzymatic activity at the specified factor, reached to 95.00 UmL<sup>-1</sup> in some cases. Partial purification of the enzyme increased its activity up to 180 UmL<sup>-1</sup>. Molecular weight determination revealed the presence of two distinct bands of about 81 and 43 KDa. [Academia Arena, 2009;1(5):89-98]. ISSN 1553-992X.

**Keywords:** Actinomycetes, *Streptomyces*, Mangrove Sediment, Cellulase, Antibiotic.

### 1. Introduction

Cellulose, a polymer of glucose residues connected by  $\beta$ -1, 4 linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature (Saha *et al.*, 2006). Cellulose is commonly degraded by an enzyme called cellulase. Cellulase is the enzyme that hydrolyzes the  $\beta$ -1, 4-glycosidic bonds in the polymer to release glucose units (Nishida *et al.*, 2007). This enzyme is produced by several microorganisms, commonly by bacteria and fungi (Immanuel *et al.*, 2006). Although a large number of microorganisms are capable of degrading cellulose, only a few of these produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose *in-vitro*. Fungi are the main cellulase producing microorganisms, though a few bacteria and actinomycetes have also been reported to yield cellulase activity. In general, bacterial cellulases are thought to be constitutively produced, while fungal cellulase is produced only in the presence of cellulose (Suto and Tomito, 2001).

A wide variety of bacteria are known for their production of extracellular hydrolytic enzymes including cellulases, with streptomycetes being the best known enzyme producers (Vinogradova and Kushnir, 2003). Within the eubacteria there is considerable concentration of cellulolytic capabilities among the predominantly aerobic order Actinomycetales (phylum Actinobacteria). Actinomycetes, one of the known cellulase-producers, have attracted considerable research interest due to its potential applications in recovery of fermentable sugars from cellulose (Jang and cheng, 2003).

The architecture of bacterial cellulases classification has always considerable functional variations. Davies and Henrissat (1995) reported that some bacterial cellulases display both modes of cellulase action, endo- and exo-. Lynd *et al.* (2002) reported a very acceptable model for synergy among different cellulases according to the region of action on the cellulose crystal as follows. Exocellulases (exoglucanases) are described as active on the crystalline regions of cellulose; whereas, endocellulases (endoglucanases) are typically active on the more soluble amorphous region of the cellulose crystal. There is a high degree of synergy seen between exoglucanases and endoglucanases, and it is this synergy that is required for the efficient hydrolysis of cellulose crystals. Some bacteria also produce intra- or extra-cellular  $\beta$ -glucosidases to cleave cellobiose and cellobioses and produce glucose to be taken up by or assimilated by the cell.

In many previous studies, *Streptomyces* species have been always reported as a source of thousands of bioactive compounds. Enzymes are one of the important products of this unusual group of bacteria. Throughout past decades, in the genus *Streptomyces*, cellulose-degrading activity has been found in some strains reported by many workers, for example; Enger and Sleeper (1965); Kluepfel *et al.*, (1986); and Spear *et al.*, (1993).

So, the aim of the current study was to continue exploring the range of bioactive compounds (cellulases in the current study) of an antibiotic producing *Streptomyces* isolate previously reported by Abdel-Shakour (2007), *Streptomyces* sp. isolate H-1. This isolate was previously isolated from mangrove sediment from Hurgada natural protectorates in Egypt, and characterized by stability over years of sub-culturing since, 2002. Also, it was characterized by producing aromatic compound very similar to the antibiotic Tubercmycin A (C<sub>17</sub> H<sub>16</sub> O<sub>2</sub> N<sub>2</sub>), active mainly against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, besides a good evidence for harboring conjugative plasmid molecule, which makes this isolate a very fit model for industrial microorganism. Conventional taxonomic methods were previously revealed that the isolate H-1 was likely a variety of *S. griseoincarnatus*. Also, identification of the isolate was assessed by using RNA polymerase  $\beta$ -subunit gene analysis, proved by Kim *et al.* (2004), and the isolate H-1 was found to has high sequence similarity (98 %) to *S. coelicolor* A3(2) followed by (93 %) sequence similarity to *S. avermitilis* MA-4680 (Abdel-Shakour, 2007).

## 2. Materials and methods

### 2.1. The microorganism used

*Streptomyces* isolate H-1 was used to detect cellulase activity in the current study. This isolate was previously isolated, identified, and investigated, as mentioned in introduction section, as to produce bioactive compounds in the work of Abdel-Shakour (2007).

### 2.2. Media used for cultivation and enzyme production

Two commercial semisynthetic cellulose substrates were used in this study, carboxymethylcellulose (CMC) and crystalline cellulose; purified cotton seed linters. The following medium was used which could be considered a modified inorganic salts starch nitrate medium into which starch was replaced by the cellulose substrates under study, CMC and crystalline cellulose of cotton linters, as soluble and insoluble cellulose substrates, respectively. The medium composed of (g/L): cellulose substrate (as the only carbon and energy source), 20; NaNO<sub>3</sub>, 2.0; K<sub>2</sub>HPO<sub>4</sub> (anhydrous basis), 1.0; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5; KCl, 0.5; FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.01 and tap water, 1000 ml. The pH value was checked & adjusted at 7 before sterilization if necessary. This medium was used for cultivation and enzyme production optimizing conditions in the form of crystalline cellulose broth which inoculated heavily with a spore suspension of the selected isolate under study and incubated aerobically (200 rpm), if otherwise stated, at 30 °C  $\pm$  2 for one week, then; the culture filtrate (about 50 ml) was assayed *in-vitro* as it contains the crude enzyme. CMC agar plates (2 % agar was added) were used for qualitative assay, while, CMC broth for *in-vitro* quantitative assay was used as mentioned below.

### 2.3. Antimicrobial activity test

The isolate was inoculated into crystalline cellulose broth medium and incubated for 7 days on a rotary shaker (200 rpm) at 30 °C. Testing the antimicrobial activity was carried out by using the classical filter paper/agar diffusion method (Cooper, 1972) after collection of supernatant and exclusion of cellulose residue by filtration. The following microbial cultures were used as test organisms: *Bacillus subtilis* (NCTC 10400) & *Staphylococcus aureus* (NCTC 7447).

### 2.4. Cellulase activity qualitative assay

The cellulase activity was determined by inoculating carboxy methyl cellulose (2 % w/v CMC) agar plates (pH 4.5) and was incubated at 30°C. After 3 & 7 days of growth, the hydrolysis zone was visualized around the culture by treating the plates with an aqueous solution of 0.1% Congo red for 15 min and then destained by washing with 1 M NaCl (Apun *et al.*, 2000 and Ariffin *et al.*, 2008). Also, the same plates were used for *in-vitro* assay using the culture filtrate of crystalline cellulose broth (2 % w/v at pH 7), where, agar wells were cut away and the filtrate was placed and allowed to diffuse for 6 hours at 30°C. In both cases, formation of a clear zone of hydrolysis indicated cellulose degradation by the produced enzyme of the tested isolate.

### 2.5. Cellulase activity quantitative assay

The cellulase activity of the same culture filtrate, after growth on crystalline cellulose broth as above in qualitative assay, was measured by determining the amount of reducing sugars liberated by using Dinitrosalicylic acid (DNS) method (Miller, 1959). Briefly, a reaction mixture composed of 0.5 ml of CMC broth, 0.5 ml of crude enzyme (culture filtrate) and 0.5 ml of 0.05 M citrate buffer pH 4.8 were

incubated for 30 minutes at 40 °C before adding 2 ml of DNS solution. The treated samples were boiled for 15 min prior to cool down in cold water bath for color stabilization. Then, the optical density was read at 540 nm against reagent blank by UV/VIS spectrophotometer. By using a calibration curve for glucose, results were interpreted in terms of enzyme activity in which one unit (U) of enzyme activity was defined as the amount of enzyme, which liberates 1  $\mu\text{mol}$  of glucose equivalent per minute, or, one unit of enzyme was expressed as 1  $\mu\text{mol}$  of reducing sugar released per min per ml, under the above assay conditions.

## 2.6. Protein determination & Biomass yield

Extracellular protein concentrations, of the same culture filtrate, after growth on crystalline cellulose broth as above in qualitative assay, were determined and expressed as mg per ml by using Lowry method with bovine serum albumin as a standard according to Lowry *et al.*, (1951). Biomass yield of the isolate under study was also measured by dry weight determination. The biomass residue was dried at 40 °C for 24h and the yield was expressed as  $\text{mg mL}^{-1}$ .

## 2.7. Enzyme production optimization

The effect of different incubation factors on the production of cellulase enzyme by the isolate under study was conducted. The variables tested were the incubation period, aerobic incubation under static and shaking conditions, incubation temperature, the pH of the culture medium, and different concentrations of the used cellulosic substrate under study (purified cotton seed linters). The above cultivation medium (crystalline cellulose broth) was used and at the end of incubation, both enzyme activity and the protein concentrations, for each culture under the tested variable were determined, besides, measuring the biomass yield as described above. The incubation under static and shaking conditions (100 & 200 rpm) at 30 °C for one week was tested first. Then, the incubation period was conducted for 3, 5, 7, 9 and 11 days. The effect of different pH values (4, 5, 6, 7 & 8), and temperature range (25, 30, 35, 40 & 45 °C) on cellulase production was conducted by incubating the selected isolate for one week (at 200 rpm & 2 % w/v C source). Also, the C source concentration of the used substrate (purified cotton seed linters) was tested from 0.5-2.5 % (w/v).

## 2.8. Enzyme production, concentration, and purification

The isolate under study was allowed to grow and produce cellulase under the determined optimum conditions. Then, the culture filtrate (about 50 ml) was collected by centrifugation at 5000 rpm for 5 min at 4 °C. The crude enzyme in the supernatant was then concentrated by ethyl alcohol precipitation. Ethyl alcohol (70 %) was added to the filtrate by percentage of 3:1 (v/v), then, allowed for precipitation for an hour and then centrifuged at 5000 rpm for 5 min at 4 °C. The precipitated enzyme was dissolved in 30 ml of sodium acetate buffer (0.2 M) at pH 5.5 and was dialyzed against the same buffer overnight at 4°C for partial purification. The obtained enzyme (about 5 ml) was concentrated against sucrose and then refrigerated at 4°C until further analysis. The partially purified enzyme was then subjected to enzyme activity and protein concentration determinations as described above, as well as, determination of molecular weight as described briefly below.

## 2.9. Determination of molecular weight

The molecular weight of cellulase enzyme obtained from the isolate under study was determined by using Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (SDS-PAGE) technique according to Sambrook *et al.*, (1989). The gel was analyzed using AlphaEaseFC 4.0 software.

## 3. Results

### 3.1. Evaluating antimicrobial and cellulolytic activities of *Streptomyces* sp. H-1

The isolate *Streptomyces* sp. H-1 was, as previously mentioned, isolated from mangrove sediment from Hurgada natural protectorates in Egypt. This isolate was previously isolated, identified, and investigated, as mentioned in introduction section, as to produce bioactive compounds in previous work of Abdel-Shakour (2007). Also, it was characterized by producing aromatic compound very similar to the antibiotic Tubermycin A, active mainly against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, when grown on starch nitrate broth medium.

In the current study, we used crystalline cellulose, in the form of cotton linters, broth medium to investigate the ability of this isolate to utilize cellulose as the only carbon source while maintaining its antimicrobial activities against the tested microbial cultures under investigation. It was found that the

results of this test confirmed the presence of the antibiotic activity of the isolate detected by the clear zones of inhibition around the filter paper discs impregnated with the culture filtrate at the end of the incubation period after seven days on a rotary shaker (200 rpm) at 30 °C. The tested microbial cultures were *Bacillus subtilis* (NCTC 10400) & *Staphylococcus aureus* (NCTC 7447), and, the diameter of the inhibition zone ranged from 1.3 to 1.7 mm.

On the other hand, the same filtrate was used to detect *in-vitro* cellulase activity of the isolate using Carboxymethylcellulose (CMC) agar plates into which agar wells were cut and the filtrate was placed and allowed to diffuse for 6 hours at 30°C. In parallel, similar plates of the same composition were first inoculated with the isolate under study to investigate cellulase enzyme production *in-vivo* after three and seven days of growth at 30°C (Figure 1). In both cases, formation of a clear zone of hydrolysis, upon addition of an aqueous solution of Congo red (0.1%) indicated cellulose degradation by the produced enzyme of the tested isolate.

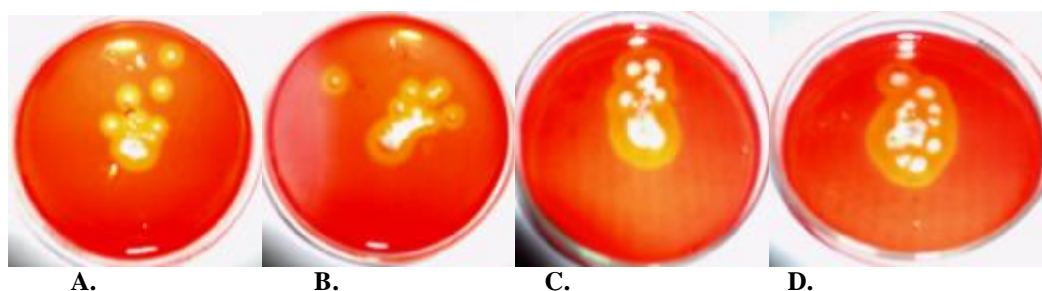


Figure 1. Growth and cellulolytic activity of the isolate *Streptomyces* sp.H-1 on 2 % CMC agar after; three days of incubation (A & B), and, seven days of incubation (C& D).

### 3.2. Cellulase enzyme production optimization by *Streptomyces* sp. H-1

The effect of different incubation factors on the production of cellulase enzyme by the isolate under study was conducted. The variables tested were the incubation period, aerobic incubation under static and shaking conditions, incubation temperature, the pH of the culture medium, and different concentrations of the used cellulosic substrate under study (cotton linters). The cultivation medium (crystalline cellulose broth), as above in qualitative assay, was inoculated and at the end of incubation, we determined the enzyme activity of the culture filtrate, by measuring the amount of reducing sugars liberated, the extracellular protein concentration, and the biomass yield for each culture under the tested variable.

Results revealed that the incubation under shaking conditions (200 rpm) was best where the recorded cellulase activity was 81.00 UmL<sup>-1</sup> and the protein concentration & the biomass yield were 0.80 & 9.30 mg mL<sup>-1</sup>, respectively (Table 1). Results also revealed that the incubation period for seven days was best where the recorded cellulase activity was 87.00 UmL<sup>-1</sup> and the protein concentration & the biomass yield were 0.89 & 9.5 mg mL<sup>-1</sup>, respectively (Table 2). On the other hand, while, results in table (3) revealed that incubation at pH value 7.00 was best where the recorded cellulase activity was 89.00 UmL<sup>-1</sup> and the protein concentration & the biomass yield were 0.95 & 9.30 mg mL<sup>-1</sup>, respectively, results in table (4) revealed that the incubation at temperature 35 °C was best where the recorded cellulase activity was 95.00 UmL<sup>-1</sup> and the protein concentration & the biomass yield were 1.10 & 9.00 mg mL<sup>-1</sup>, respectively. Finally, results revealed that growth of the isolate under study was best on crystalline cellulose (cotton linters) concentration of 2.0 % (w/v) where the recorded cellulase activity was 85.00 UmL<sup>-1</sup> and the protein concentration & the biomass yield were 1.00 & 9.40 mg mL<sup>-1</sup>, respectively (Table 5).

Table 1. Effect of aerobic incubation under static and shaking conditions of the culture medium on cellulase production by the isolate *Streptomyces* sp. H-1

Incubation condition	Enzyme activity U mL <sup>-1</sup>	Protein concentration mg mL <sup>-1</sup>	Biomass yield mg mL <sup>-1</sup>
Static	27.00	0.30	2.70
Shaking (100 rpm)	49.00	0.47	6.50
Shaking (200 rpm)	81.00	0.80	9.30

Table 2. Effect of the incubation period of the culture medium on cellulase production by the isolate *Streptomyces* sp. H-1

Incubation period days	Enzyme activity U mL <sup>-1</sup>	Protein concentration mg mL <sup>-1</sup>	Biomass yield mg mL <sup>-1</sup>
3	25.00	0.28	3.00
5	53.00	0.57	6.20
7	87.00	0.89	9.50
9	77.00	0.84	9.00
11	73.00	0.84	9.20

Table 3. Effect of different initial pH values of the culture medium on cellulase production by the isolate *Streptomyces* sp. H-1

Initial pH value	Enzyme activity U mL <sup>-1</sup>	Protein concentration mg mL <sup>-1</sup>	Biomass yield mg mL <sup>-1</sup>
4	7.00	0.07	0.90
5	24.00	0.28	2.70
6	51.00	0.60	5.90
7	89.00	0.95	9.30
8	81.00	0.90	8.80

Table 4. Effect of different incubation temperatures of the culture medium on cellulase production by the isolate *Streptomyces* sp. H-1

Incubation temperature °C	Enzyme activity U mL <sup>-1</sup>	Protein concentration mg mL <sup>-1</sup>	Biomass yield mg mL <sup>-1</sup>
25	59.00	0.67	5.50
30	79.00	0.98	8.00
35	95.00	1.10	9.00
40	73.00	0.92	7.70
45	49.00	0.59	4.80

Table 5. Effect of different cellulose concentrations of the culture medium on cellulase production by the isolate *Streptomyces* sp. H-1

Cellulose concentration % (w/v)	Enzyme activity U mL <sup>-1</sup>	Protein concentration mg mL <sup>-1</sup>	Biomass yield mg mL <sup>-1</sup>
0.5	18.00	0.20	1.90
1.0	45.00	0.50	4.70
1.5	70.00	0.85	8.20
2.0	85.00	1.00	9.40
2.5	68.00	0.80	7.80

### 3.3. Characterization of partially purified cellulase produced by *Streptomyces* sp. H-1

The isolate under study was allowed to grow and produce cellulase under the determined optimum conditions depicted in table (6). Then, the culture filtrate, containing the crude enzyme was collected and subjected to partial concentration and purification including ethyl alcohol precipitation, centrifugation, and dialysis against sucrose. The partially purified enzyme was then subjected to enzyme activity and protein

concentration determinations, as well as, determination of molecular weight using SDS-PAGE technique.

The results obtained revealed that the activity of the partially purified cellulase produced by the isolate under study had increased by about factor of two where the recorded activity was  $180 \text{ U mL}^{-1}$  while the protein concentration determined reached to  $1.70 \text{ mg mL}^{-1}$ . Also, the approximate molecular weight for the enzyme was determined from a constructed standard curve for the known protein marker against the distance migrated in mm. Results in figure (2) showed that the cellulase enzyme had separated into two distinct bands of approximate molecular weights of 81 and 43 KDa.

Table 6. The optimum cultural conditions for cellulase-production by *Streptomyces* sp. H-1

Optimum conditions	Cellulase activity ( $\text{U mL}^{-1}$ )
Shaking incubation (200 rpm)	81.00
Incubation period (7 days)	87.00
Initial pH value (7.00)	89.00
Incubation temperature ( $35^\circ\text{C}$ )	95.00
Cellulose concentration (2.00 % w/v)	85.00

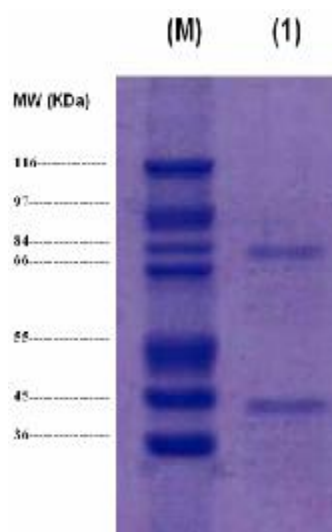


Figure 2. Molecular weight determination of cellulase produced by *Streptomyces* sp.H-1 using SDS-PAGE; (M) is the protein marker & (1) is the enzyme sample.

#### 4. Discussion

Aerobic cellulose degraders, both bacterial and fungal, utilize cellulose through the production of substantial amounts of extracellular cellulase enzymes that are freely recoverable from culture supernatants (Schwarz, 2001). The individual enzymes often display strong synergy in the hydrolysis of cellulose. It is also notable that most aerobic cellulolytic bacterial species common in soil are classified within genera well known for secondary (non-growth-associated) metabolism, including the formation of distinct resting states and/or production of antibiotics (*Bacillus*, *Streptomyces*, and *Micromonospora*) and other secondary metabolites. While antibiotic production in cellulolytic species has not been systematically investigated, production of such compounds might provide additional selective fitness to compensate for their rather modest maximum growth rate on cellulose. An ability to form resting states relatively resistant to starvation or other environmental insult also provides a selective advantage in nature (Lynd *et al.*, 2002).

Ulrich and Wirth (1999) conducted a phylogenetic study of culturable cellulolytic soil bacteria diversity across an agricultural encatchment and found that the ratio of actinomycetes within total isolates ranged from 0.73 to 0.94, and also, based on 16S rDNA sequence analysis, isolates of the dominant as well as the specific pattern groups could be assigned to the genus *Streptomyces*. Previously, LI (1997) has isolated an actinomycete strain, which decomposes cellulose, and was identified as a member of the genus *Streptomyces* on the basis of morphological characteristics and chemo-type of the cell wall. Sakon *et al.*, (1997) reported that the family 9 cellulase of aerobic *Thermomonospora fusca* has provided strong

evidence for enzymes that can exhibit both endoglucanase and exoglucanase activities. Veiga *et al.*, (1983) reported the presence of actinomycetes capable of cellulose decomposition from marine sediments.

The current study aim was to evaluate the antimicrobial and cellulolytic activities of *Streptomyces* sp. H-1, previously isolated from mangrove sediment from Hurgada natural protectorates in Egypt while growing on the used crystalline cellulose substrate (cotton linters). This isolate was identified and investigated previously by Abdel-Shakour (2007). Also, it was characterized by producing aromatic compound very similar to the antibiotic Tubercycin A. The use of commercial cotton crystalline cellulose was to approach the nature of cellulosic biomass which is more complex than that of pure celluloses. The isolate was able to utilize crystalline cellulose in broth medium as the only carbon and energy source and also produced its antibiotic active against *Bacillus subtilis* and *Staphylococcus aureus*. Growth on insoluble crystalline cellulose supported the hypothesis that this isolate produce a powerful cellulase enzyme system *In-vivo* composed of endocellulase, exocellulase, and  $\beta$ -glucosidase in order to obtain glucose.

The screening process of cellulolytic bacteria usually conducted using the Congo red test. Since the sole carbon source in the agar is CMC, therefore, the result of the test is a strong evident that cellulase was produced in order to degrade cellulose (Lynd *et al.*, 2002; Wang *et al.*, 2008).

In our study, the clear zone around *Streptomyces* colonies after staining with Congo red indicated the hydrolysis of CMC, despite absence of crystallinity, as a result of cellulases produced and this phenomenon has been reported also by Abdel-Nasser and Ahmed (2007). Moreover, it was reported that *Geobacillus* strain was capable in hydrolyzing cellulose (Miyazaki *et al.*, 2007). As detected from Congo red method, the isolated strain had endoglucanase activity, one of the enzymes required for conversion of cellulose to glucose as reported by Sirisena and Manamendra (1995). According to Ariffin *et al.*, (2008) cellulolytic bacteria, *Bacillus pumilus* EB3 was successfully isolated and produced clear zone around the colony after staining with Congo red on CMC agar.

So, in our study, both *In-vivo* & *In-vitro* cellulase assay for detecting endocellulase, exocellulase, and  $\beta$ -glucosidase activity was conducted by using the culture filtrate (crude enzyme) onto agar wells cut in CMC agar plates. In parallel, similar plates of the same composition were first inoculated with the isolate under study to investigate cellulase enzyme production. In both cases, formation of a clear zone of hydrolysis, upon addition of an aqueous solution of Congo red indicated cellulose degradation by the produced enzyme of the tested isolate. However, the result of this test confirm only the presence of endocellulase or endoglucanase only specially when the enzyme tested *In-vitro*.

Cellulase enzyme production optimization by *Streptomyces* sp. H-1 was conducted. The variables tested were the incubation period, aerobic incubation under static and shaking conditions, incubation temperature, the pH of the culture medium, and different concentrations of the used cellulosic substrate under study (cotton linters). The cultivation medium (crystalline cellulose broth), as above in qualitative assay, was inoculated and at the end of incubation, we determined the enzyme activity of the culture filtrate, by measuring the amount of reducing sugars liberated using CMC broth as a substrate, the extracellular protein concentration, and the biomass yield for each culture under the tested variable. However, the used enzyme-substrate (CMC) will confirm endocellulase activity definitely, but, provide a little about exocellulase and  $\beta$ -glucosidase, since both exocellulose and cellobiose give positive results in this test as they contain reducing ends as glucose. At least, we report that, the isolate must produced the three set of enzymes as it was grown on crystalline cellulose broth. Hence, in other studies, cellulase activity was determined by filter paper method of Stephen *et al.*, (2003) as this method is a combined assay for endo- and exo-glucanases *In-vitro*.

Our results revealed that the aerobic incubation under shaking conditions (200 rpm) was best where the recorded cellulase activity was 81.00 U mL<sup>-1</sup> and the protein concentration & the biomass yield were 0.80 & 9.30 mg mL<sup>-1</sup>, respectively. Results also revealed that the incubation period for seven days was best where the recorded cellulase activity was 87.00 U mL<sup>-1</sup> and the protein concentration & the biomass yield were 0.89 & 9.5 mg mL<sup>-1</sup>, respectively. On the other hand, our results revealed that incubation at pH value 7.00 was best where the recorded cellulase activity was 89.00 U mL<sup>-1</sup> and the protein concentration & the biomass yield were 0.95 & 9.30 mg mL<sup>-1</sup>, respectively, and also the incubation at temperature 35 °C was best where the recorded cellulase activity was 95.00 U mL<sup>-1</sup> and the protein concentration & the biomass yield were 1.10 & 9.00 mg mL<sup>-1</sup>, respectively. Finally, results revealed that growth of the isolate under study was best on crystalline cellulose concentration of 2.0 % (w/v) where the recorded cellulase activity was 85.00 U mL<sup>-1</sup> and the protein concentration & the biomass yield were 1.00 & 9.40 mg mL<sup>-1</sup>, respectively.

Similar studies were conducted by many authors with variations, for example, Theberge *et al.*, (1992) reported that the optimum pH for endoglucanase from a strain of *Streptomyces lividans* was 5.50. Solingen *et al.*, (2001) reported that an alkaline novel *Streptomyces* species isolated from east African soda lakes have an optimal pH of 8.00. McCarthy (1987) reported that the optimal temperature for cellulase activity in the range of 40-55 °C for several *Streptomyces* species including *S. lividans*, *S. flavogrisus*, and *S. nitrosporus*. Jang and Chen (2003) described a CMCase produced by *Streptomyces* T3-1 with optimum temperature 50 °C, whereas Schrempf and Walter (1995) described a CMCase production by *S. reticuli* at an optimum temperature 55 °C. It has been reported that the biosynthesis of cellulase is induced during growth on cellulose or other cellulose derivatives (Fernandez- Abalose *et al.*, 1997; Godden *et al.*, 1989). In all cases, it has been found that it is essential to keep the required nutrients at low level to insure maximum accumulation of fermentation products (Priest, 1984). However, the purpose of Jaradat *et al.*, (2008) study was to determine the influence of growth conditions and medium composition on the cellulase enzyme production by *Streptomyces* sp. (Strain J2) and he reported that the highest crude enzyme activity (432 U/L) was observed after 3 days of incubation at pH 7.00 and 60 °C in CMC broth that was supplemented with 0.5% glucose, 0.2% starch, and 0.2% NH<sub>4</sub>Cl.

Finally, characterization of partially purified cellulase produced by *Streptomyces* sp. H-1 under the determined optimum conditions revealed that the activity had increased by about one fold where the recorded activity was 180 U mL<sup>-1</sup> and protein concentration determined reached to 1.70 mg mL<sup>-1</sup>. Also, the approximate molecular weight for the cellulase enzyme was determined and the enzyme had separated into two distinct bands of 81 and 43 kDa in size, despite the subsidiary subunits constituents of each band. The determined molecular weights of the cellulase of our isolate under study were in agreement with the many other studies of bacterial cellulases molecular weights range determinations, see (Lynd *et al.*, 2002) for reviews.

Before closing comments, we must address here that novel cellulase producing bacteria still need more research for discovery of unusual enzyme systems and determine their efficiency especially for the industrial use applications. For example, a bacterial strain B39 previously isolated and identified through 16S rRNA gene sequencing and phylogenetic analysis to be a novel cellulose-degrading *Paenibacillus* sp. strain with a high-molecular weight (148 kDa) cellulase was discovered. The endocellulase or CMCcase activity of the newly isolated cellulase was much higher than the activity on Avicel or filter paper and this cellulase was found to have maximum CMCcase activity at 60°C, pH 6.50. Due to the promising thermostability and slight acidic tolerance of this enzyme, it has good potential for industrial use in the hydrolysis of soluble cellulose as well as activity on microcrystalline sources of cellulose (Wang *et al.*, 2008).

Similarly, a multifunctional enzyme was found to be produced by *Teredinibacter turnerae* T7902, which is a bacterial symbiont isolated from the wood-boring marine bivalve *Lydrodus pedicellatus*. This CelAB was found to have two catalytic and two carbohydrate-binding domains. It binds both cellulose and chitin and possesses cellobiohydrolase and beta-1,4(3) endoglucanase activity allowing it to degrade multiple complex polysaccharides. This enzyme is marginally acid-tolerant at an optimum pH of 6 and mesophilic with a temperature optimum of 42°C. Additionally, this enzyme was able to reduce viscosity of CMC approximately 40% after 25 minutes, displaying promising characteristics for bio-fuel industry (Ekborg *et al.*, 2007).

In conclusion, the results of this study revealed the promising of using our isolate, *Streptomyces* sp.H-1, in *In-vivo* crystalline cellulose degradation and antibiotic production effectively. The development of our enzyme for *In-vitro* industrial use still needs more research, especially the physiology of the organism itself, apart from studies on the effect of growth conditions on enzyme production and improving the yield. Also, this will be feasible especially through development of rapid and reliable methods for screening of cellulases from microorganisms for industrial use. Finally, the biotechnological approaches to developing practical processes for the conversion of cellulose to fuels and commodity chemicals involve, to a greater extent, the microbial cellulose utilization approach.

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