academicJournals

Vol. 10(13), pp. 410-419, 7 April, 2016
DOI: 10.5897/AJMR2016.7954
Article Number: 13142B457910
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Optimization of cellulase production for *Bacillus* sp. and *Pseudomonas* sp. soil isolates

Arusha P. Nandimath[1,3], Kiran R. Kharat[2], Shanti G. Gupta[3] and Arun S. Kharat[1]*

[1]Department of Biotechnology, Dr. Babasaheb Ambedkar Marathwada University, Subcampus Osmanabad, MS 413501, India.
[2]Department of Biotechnology, Deogiri College, Aurangabad, MS 431001, India.
[3]Government Institute of Forensic Sciences, Aurangabad, MS 431004, India.

Received 7 February, 2016; Accepted 15 March, 2016

This investigation deals with optimizing the cellulase producing bacterial isolates for better enzyme yield. Cellulose degrading bacteria were isolated from garden soil (Aurangabad, MS, India). Amongst 40 different bacterial isolates, two of the bacterial isolates, 2b and 38b, were processed for preliminary identification with morphological, cultural and biochemical characterization. Based on these studies, strain 2b was qualified to belong to *Pseudomonas* sp. and strain 38b was qualified to belong to *Bacillus* sp. Optimization of the fermentation medium for maximum cellulase production was carried out for both strains of 2b and 38b. The culture conditions such as: pH, temperature, substrate concentration and incubation time were optimized. The optimum conditions found for cellulase production were 30°C at pH 5. Studies on partially purified cellulase, a high cellulolytic activity was observed in *Pseudomonas* sp. The Km 22.11 and 14.36 were obtained for cellulase from 2b and 38b, respectively. The Vmax was found to be 1 and 1.121 mmol (min mg)$^{-1}$ for cellulase from 2b and 38b , respectively.

Key words: *Bacillus* sp., cellulase production, pH, *Pseudomonas* sp.

INTRODUCTION

Cellulose is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the biosphere (Jarvis, 2002; Zhang and Lynd, 2004). Plants are the most abundant source of cellulose and are found as microfibrils (“2-20 nm” in diameter and “100-40,000 nm” in length). Cellulase is one of the enzymes produced mainly by fungi, bacteria and protozoans that catalyze cellulolysis (the decomposition of cellulose and of some related polysaccharides). In spite of the high growth rates of bacteria, they do not have enough cellulase production as with the fungi. Though several microorganisms are now known as cellulase producers, a relatively few numbers of fungi and bacteria are known to produce the enzyme in high levels. So far, most of the studies have been focused on the cellulase producing fungi (Callow et al., 2016; Sharma et al., 2015; Saini et al., 2015; Lan et al., 2013), bacteria (Shanmugapriya et al., 2012; Assareh

*Corresponding author. E-mail: arunkharat2007@gmail.com. Tel: +91-240-2403227. Fax: +91-240-2403335.

et al., 2012; Rastogi et al., 2010) and actinobacteria (Cirigliano et al., 2013; Sarita et al., 2013). However, the application of bacteria in producing cellulase is not widely used (Bhat, 2000). The cellulolytic property of some bacterial genera such as *Cellulomonas, Cellvibrio, Pseudomonas* (Nakamura et al., 1982), *Bacillus* and *Micrococcus* has been reported (Immanuel et al., 2006).

Enzyme production is closely controlled in micro-organisms and to improve its productivity, these controls can be ameliorated. Cellulase yields appear to depend upon a complex relationship involving a variety of factors such as inoculum size, pH value, temperature, presence of inducers, medium additives, aeration and growth time (Immanuel et al., 2006). Enormous amounts of agricultural, industrial and municipal cellulosic wastes are accumulating or used inefficiently due to their high cost towards utilization processes (Lee et al., 2008).

Therefore, this problem is arising as a topic of considerable economic interest to develop processes for the effective treatment and utilization of cellulosic wastes as inexpensive carbon sources. Cellulose containing wastes may be agricultural, urban, or industrial in origin, and in addition, sewage sludge might also be considered as a source of cellulose since its cellulosic content provides the carbon for methane production in the anaerobic digestion of the sludge. This study primarily focused on obtaining an efficient degradation of cellulose from garden soil bacteria and this was achieved by optimizing the physical conditions (pH, temperature, etc) and nutritional requirements (substrate concentration) with respect to time.

MATERIALS AND METHODS

Isolation and screening of bacteria

Isolation of microorganisms from garden soil

The cellulolytic bacteria were isolated from garden soil. The damp and wet garden soil was used for isolation of cellulose degrading bacteria. The CMC medium was used for the enrichment and isolation of microorganisms. About one gram soil was inoculated into total 10 ml CMC broth (w/v), incubation for enrichment was carried out for 15 days at room temperature. After 15 days of incubation, turbid culture was serially diluted (10 fold) in physiological saline (sterilized 0.85% NaCl). To obtain single colonies of cellulose degraders, serial dilutions were spread plate inoculated onto the CMC agar. At the same time, enriched suspension was directly streak inoculated onto separate CMC agar plate. These plates were then incubated at room temperature for the appearance of single colonies. To visualize the hydrolysis zone (cellulose production), the plates were flooded with an aqueous solution of 0.1% Congo red for 15 min and washed with 1 M NaCl (Apun et al., 2000).

To indicate the cellulolytic activity of the organisms, diameter of hydrolytic zone around the growing colony on CMC agar was measured. Isolate qualifying for cellulose production was then used for quantification of cellulose activity in liquid medium. The cellulase activity of each culture was measured by determining the amount of reducing sugars liberated by using a di-nitro salicylic acid (DNSA) colorimetric method (Miller, 1959). Bacterial isolates 2b and 38b exhibiting relatively high enzyme activity of other isolates were selected for optimization of cellulose production.

Bacterial identification

The bacterial isolates were presumptively identified by means of morphological examination and some biochemical characterizations. The parameters investigated included colonial morphology, Gram's reaction, endospore formation, catalase production, Voges Proskauer (VP) reaction, indole production, starch hydrolysis, citrate utilization and gelatine hydrolysis. The results were compared with Bergey's Manual of Determinative Bacteria (Buchanan et al., 1974).

Production of cellulase

The bacterial isolates 2b and 38b were used in the cellulase production and optimization studies. The CMC broth (50 ml) in 500 ml flask was inoculated and the cellulase production was carried out. The CMC broth used was a minor modified version of ATCC medium 2270. In brief, for 1 L medium $(NH_4)2SO_4$ 1.0 g, $MgSO_4$ x $7H_2O$ 1.0g, $CaCl_2$ x $2H_2O$ 1.0 g, $FeCl_3$ 0.2 g, K_2HPO_4 (filter) 1.0 g, Casitone 2.0 g, carboxymethyl cellulose 15.0 g (Agar 15.0 g), was digested in double distilled water, 900 ml. Upon autoclaving, the pH was adjusted by supplementing with required sterile KH_2PO_4 solution and sterile double distilled water to make up 1 L. After 6 days incubation at room temperature, the cellulase was recovered in cell free culture supernatant by centrifugation at 1000 rpm for 25 min. The crude enzyme was extracted with 0.025 M sodium citrate buffer of pH 5.6 at 4°C and was used further in enzyme kinetics study.

Enzyme assay

For the assay, crude enzyme (2 ml) was mixed with 1% (w/v) cellulose in 0.05 mM sodium citrate buffer Ph 5. Reaction volume was made up to 5 ml by adding deionised water. The reaction mix was incubated at 30°C for 2 h. The reducing sugar product was determined by the DNSA method. Amount of released reducing sugar was estimated by plotting optical density on standard plot obtained by performing DNSA reaction on various glucose concentrations.

Process optimization for maximum cellulase production

pH

The growth medium containing the optimum concentration of substrate and carbon source was used. The pH of the broth was optimized by performing the reaction at different pH. Both 2b and 38b isolates were allowed to grow in media of different pH: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. At the end of incubation period, a cell-free culture supernatant was used as the source of enzyme.

Temperature

Production medium at pH 5 was inoculated with overnight grown bacterial strain. The broth was incubated at different temperatures of 25, 30, 35 and 40°C for 24 h. At the end of incubation period, the cell-free culture supernatant was used as the source of enzyme.

Substrate

Production medium at pH 5 was inoculated with overnight grown bacterial strain. The substrate concentration: 0.5, 1.0, 1.5, 2.0 and

4.0 mg/ml were used in separate flasks and incubated at 30°C for 24 h. At the end of incubation period, the cell-free culture supernatant was used as the source of enzyme.

RESULTS

Screening of isolates for the enzyme activity

About 40 bacterial isolates exhibiting cellulose degradation were isolated. Results shown in Table 1 and Figure 1 denote qualitative ability of these isolates needed for cellulase production. It is clear from the table that isolate 2b and 38b were found to be promising towards cellulase production ability in comparison with other isolates from this study and hence optimization for the enzyme production was studied with these two isolates, only.

Identification of bacterial isolates

Once it was noticed that amongst 40 different bacteria producing cellulase, the 2b and 38b strains were promising and we sought to address their preliminary characterization. Studies were performed to understand the morphological, cultural and characteristics and the results are shown in Table 2. Based on morphological, cultural and biochemical characters, the isolate 2b and 38b were putatively identified as *Pseudomonas* sp. and *Bacillus* sp. respectively.

Determination of enzyme activity

Results shown in Figure 2 show that isolate 2b and 38b were found to be active for cellulolytic activity after 40 min of reaction incubation at room temperature and at pH 5. However, the optimum reaction rate was observed after 60 min of incubation. ~1 and 0.9 µm/min/mg enzyme specific activity was found in crude extracts for 2b and 38b, respectively. As optimum enzyme activity was noticed with 60 min incubation, it was considered as optimum time for the reactions in further experiments.

Optimization of pH for the production of cellulase

Both 2b and 38b isolates were found to produce maximum enzyme activity at 60 min, we then sought to identify optimum pH for the cellulase production. Towards this, the pH of the broth was optimized by using the reaction buffer of different pH. Both 2b and 38b isolates were allowed to grow in media of different pH: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. Observations depicted in Figure 3 indicate that crude cellulase from 2b and 38b was found to be more active at pH 5.0. The pH 5 of growth medium and reaction mix was used in our future experiments. Our results are in agreement with the finding of other workers (Chantawannakul et al., 2002; Abdel-Mawgoud et al., 2008) who also used *Bacillus subtilis* as the model organism.

Table 1. Ability to produce cellulase, identified with the help of hydrolysis zone.

S/N	Bacterial isolates	Cellulase activity
01	1a	+
02	1b	-/+
03	2a	+
04	2b	++++
05	4a	+
06	4b	+
07	6	-
08	7a	-
09	7b	-
10	9	-
11	11	-
12	12a	-
13	12b	-
14	13	-
15	14a	-
16	14b	-
17	14c	-
18	15	-
19	16	+/-
20	21	++
21	22a	-
22	22b	-
23	27	+
24	28	+++
25	29a	+
26	29b	+++
27	30a	+
28	30b	+
29	31	-
30	32	-
31	33	++
32	36a	-
33	36b	-
34	36c	-
35	38a	+
36	38b	++++
37	38c	+
38	39	+
39	40a	+
40	40b	+++

- Negative for cellulase production, +, ++ and ++++ qualitative activity.

Optimization of temperature for the cellulase production

In the above experiment, it was shown that cellulase

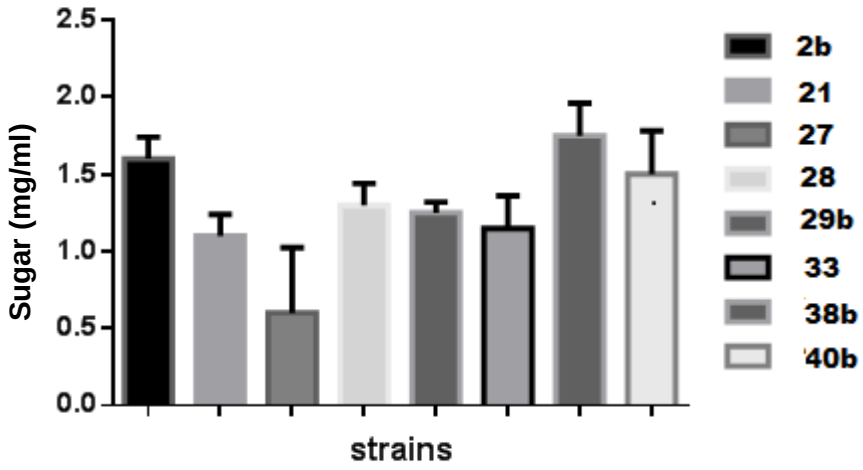

Figure 1. Screening of bacterial isolates for the production of cellulase. Total residual sugar was estimated from the growth medium of the bacterial. The cellulase activity was determined by DNSA method. Sugar concentration, 1.2 mg/ml was considered significant for the positive selection of the isolate.

Table 2. Biochemical characterization of cellulase producing bacteria.

S/N	Biochemical test	*Pseudomonas* sp.2b	*Bacillus* sp.38b
1	Grams nature	Gram negative rods	Gram positive rods
2	Motility	Motile	Motile
3	Indole production	Positive	Negative
4	Methyl Red	Negative	Positive
5	VogesProskauer	Positive	Positive
6	Citrate Utilization	Positive	Negative
7	Oxidase	Positive	
8	Endosopre formation	Negative	Positive, central non bulging spore
9	Catalase	Positive	Positive
10	Gelatin Hydrolysis	Positive	Positive
11	Starch Hydrolysis	ND	Positive
12	Urease	Positive	Negative
13	Nitrate Reduction	ND	ND
14	H_2S Production	Negative	Negative
15	Carbohydrate fermentation tests	-	-
a	Arabinose	Negative	Positive
b	Xylose	Negative	Positive
c	Maltose	Negative	Positive
d	Sucrose	Negative	Positive
e	Mannitol	ND	Positive
f	Glucose	Positive	Negative
g	Lactose	Negative	Negative

activity was found to be produced better within 60 min and at pH 5.0. The authors sought to address the optimum temperature for the production of cellulose, enzyme activity was recorded at different temperatures. Results shown in Figure 4 depicts tha both 2b and 38b strains produced maximum cellulase production at 30°C. The 30°C incubation temperature was thus used for the ES reactions in further experiments. The temperature was found to influence extracellular enzyme secretion, possibly by changing the physical properties of the cell

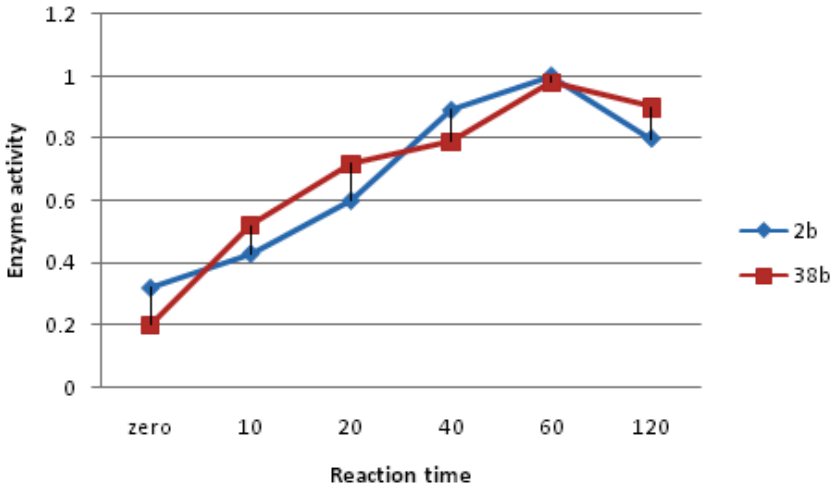

Figure 2. The enzyme specific activity of cellulase (µm/min/mg) vs. time. 2b and 38b were used for the cellulase production. The enzyme specific activity of isolated enzyme was determined at different incubation time. The optimum reaction rate was observed at 60 min incubation. Measurements were made in triplicate, and standard bars represent the standard deviation.

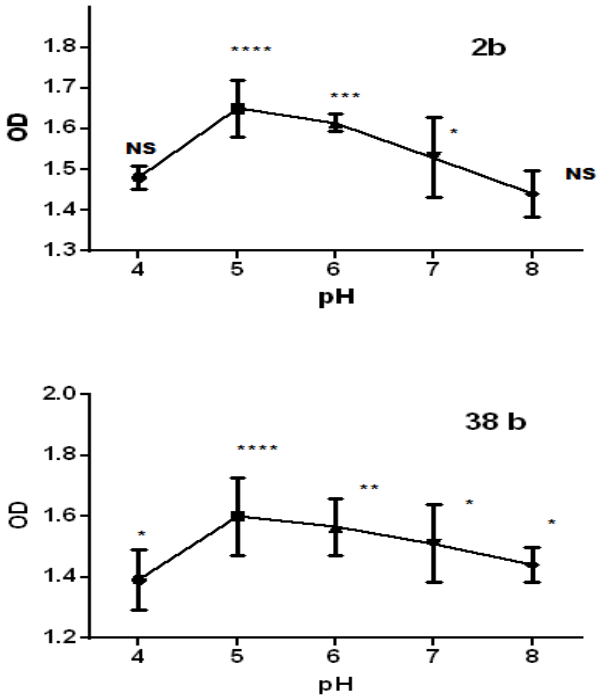

Figure 3. The production of cellulase at optimum pH. 2b and 38b were used for the cellulase production. The production of cellulase was determined at different pH. The pH of the broth was optimized by using the reaction buffer of different pH. Both 2b and 38b isolates were allowed to grow in media of different pH: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. Measurements were made in triplicate, and standard bars represent the standard deviation. The Student's two-tailed t-test was used to determine statistical significance of the differences between enzyme activities at different time interval. Error bars indicate SD (set as 100%; n = 3; ****P <0.0001, ***P <0.001, **P <0.01, *P < 0 .05, ns-not significant).

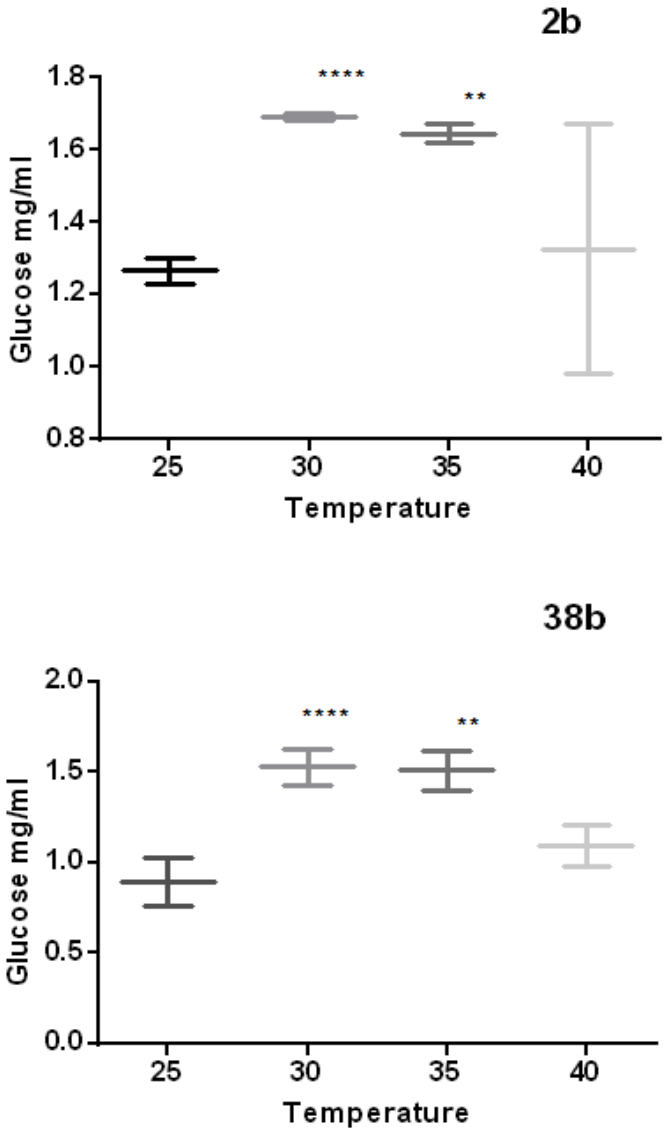

Figure 4. The optimization of temperature for the production of cellulase. 2b and 38b were used for the cellulase production. The enzyme production was recorded at different temperatures. Results depict that 2b and 38b strains produced maximum cellulase production at 30°C. The optimum reaction rate was observed at 30°C and pH 5. Measurements were made in triplicate, and standard bars represent the standard deviation. The Student's two-tailed t-test was used to determine statistical significance of the differences between enzyme activities at different time interval. Error bars indicate SD (set as 100%; n = 3; ****P <0.0001, ***P <0.001, **P <0.01, *P < 0 .05, ns-not significant).

membrane. This observation is in agreement with earlier report on *Bacillus subtilis* by Jansova et al. (1993).

Incubation time and the cellulase production

After optimizing reaction conditions for enzyme activity, the authors sought to grow strain 2b and 38b in growth medium and study the enzyme production with reference to incubation time. Crude cell free supernatant were collected every 24 h until 120 h incubation. It is evident from the Figure 5 that in case of strain 2b significant enzyme production was detected at the end of 24 h and slight increase was noticed at 48 h incubation. Further

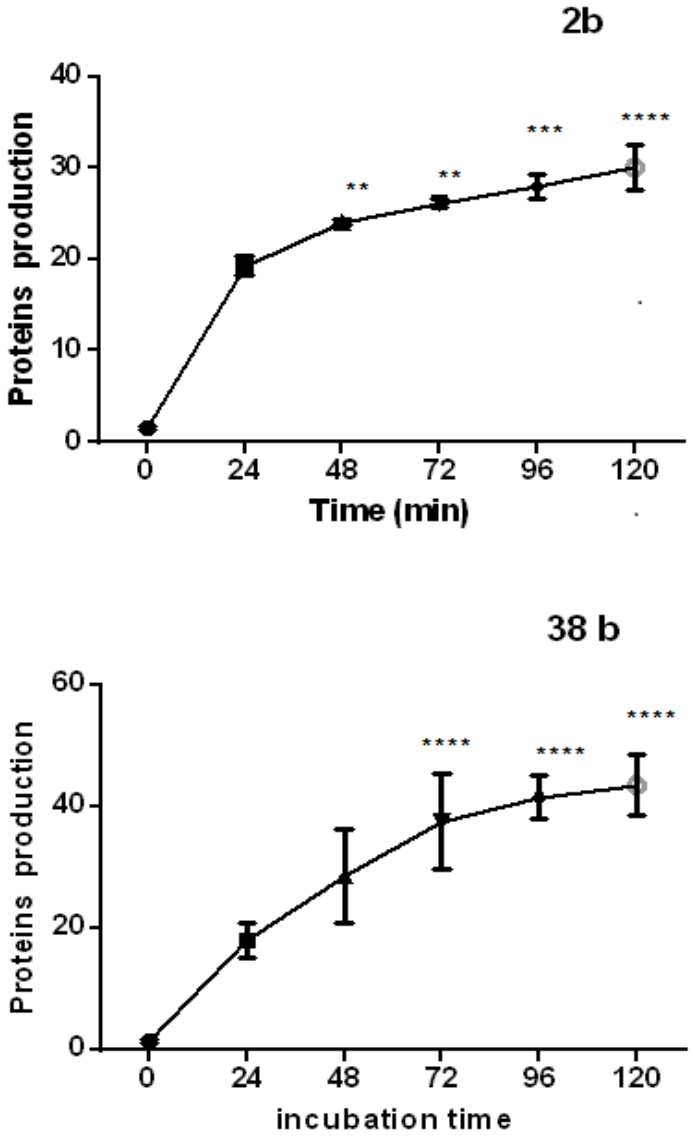

Figure 5. Cellulase production at various time durations. 2b and 38b were used for the cellulase production. The enzyme quantity was measured at various time point. Maximum protein production was observed at 120 min after incubation. Measurements were made in triplicate, and standard bars represent the standard deviation. The Student's two-tailed t-test was used to determine statistical significance of the differences between enzyme activities at different time interval. Error bars indicate SD (set as 100%; n = 3; ****P <0.0001, ***P <0.001, **P <0.01, *P < 0 .05, ns-not significant).

incubation did neither increased the enzyme production nor was it affected (Figure 5). On the other hand, strain 38b produced enzyme at 24 h time point and continued to exhibit elevated enzyme activity until last time point, 120 h. However, increased enzyme production by 38b strain beyond 48 h was not significant enough (Figure 5). The optimum reaction rate was observed after 24 h incubation.

Cellulase kinetic studies

Effect of substrate concentration on cellulase activity

The crude cellulase obtained from 2b and 38b strains was found to be active at 30°C and at pH 5. The rate of reaction was found to increase with increase in the substrate concentration, shown in Figure 6. The maximum

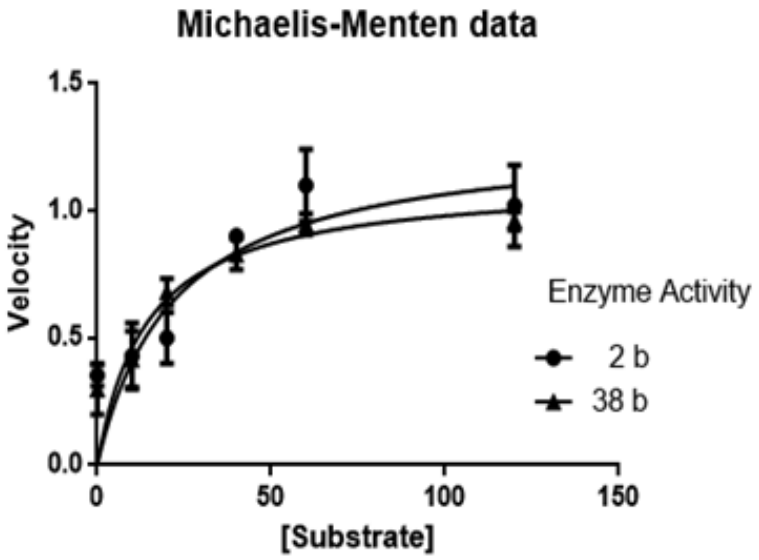

Figure 6. Enzyme kinetics of cellulase isolated from 2b and 38b. Cellulase from 2b and 38b were used for the Michaelis-Menten kinetics. The enzyme specific activity of isolated enzyme was determined at various concentrations of substrates. The Km was determined by using the GraphPad Prism 6.01. The Km 22.11 and 14.36 was obtained for cellulase from 2b and 38b, respectively. The Vmax was found to be 1.3 and 1.121 mmol(min mg)$^{-1}$ for cellulase from 2b and 38b, respectively. Measurements were made in triplicate, and standard bars represent the standard deviation.

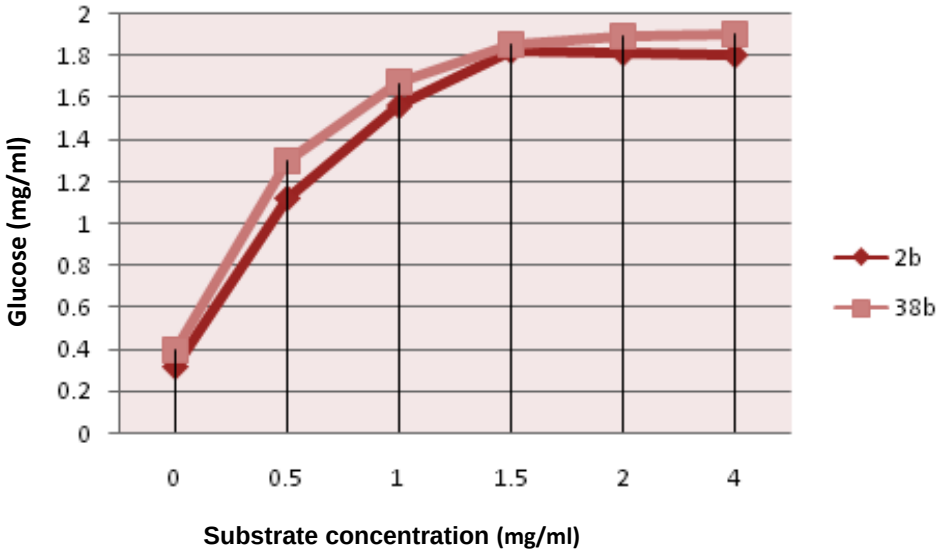

Figure 7. Optimization of substrate concentration for the cellulase. 2b and 38b were used for the cellulase production. The enzyme specific activity of isolated enzyme was determined at various concentrations of substrates. Measurements were made in triplicate, and standard bars represent the standard deviation.

activity at 30°C, pH 5, with 60 min was found with 1.5 mg substrate concentration shown in Figure 7. The Km was estimated with the use of GraphPad Prism 6.01. The Km for strains 2b and 38b was 22.11 and 14.36, respectively. The Vmax was found to be 1.3 and 1.121 mmol/min/mg for the cellulase enzyme from 2b and 38b, respectively.

Maximum enzyme activity at 48 h was found with 1.5 mg substrate concentration in cell free supernatant of 2b and 38b, respectively.

DISCUSSION

Treatment of cellulose by cellulolytic enzymes for practical purposes has attracted the continuing interest of biotechnologists. In this study, the production of cellulase from bacterial isolates was optimized. The production of cellulase from *Pseudomonas* sp. 2b and *Bacillus* sp. 38b strains was achieved.

Lignocellulosic biomass, (plant biomass), is a great potential resource for the production of biofuels because it is largely abundant, inexpensive and production of such resources is environmentally sound. Agricultural residues are a great source of lignocellulosic biomass which is renewable, chiefly unexploited, and inexpensive. Such resources include: leaves, stems and stalks from sources such as corn fibre, corn stover, sugarcane bagasse, rice hulls, woody crops and forest residues. Also, there are multiple sources of lignocellulosic waste from industrial and agricultural processes, e.g., citrus peel waste, sawdust, paper pulp, industrial waste, municipal solid waste and paper mill sludge. Both fungi and bacteria have been exploited for their abilities to produce a wide variety of cellulases and hemicellulases (Zhang and Lynd, 2004; Bhat, 2000; Nakamura et al., 1982; Sethi et al., 2013). Most emphasis has been laid on the use of fungi because of their capability to produce copious amounts of cellulases and hemicellulases which are secreted to the medium for easy extraction and purification.

Secondly, bacterial glycoside hydrolases are often more complex and expressed as multi-enzyme complex providing increased function and synergy. Most importantly, bacteria inhabit a wide variety of environmental and industrial niches, which produce cellulolytic strains that are extremely resistant to environmental stresses (Nakamura et al., 1982; Immanuel et al., 2006).

Further studies were in progress in the purification and application of cellulase in different commercial fields. The purified cellulase can be used for various purposes in detergent industries, food industries and pharmaceutical industries. The high activity and stability of cellulase enzymes between neutral to alkaline pH and high temperature will be of use in various industrial and biotechnological applications.

These results are close to those of Bakare et al. (2005) who found that the cellulase enzyme produced by *Pseudomonas fluorescence* was activated from 30 to 35°C showing the optimum temperature at 35°C. Ray et al. (2007) reported that minimum cellulase yield was observed when fermentation was carried out at 45°C, while maximum yield was obtained at 40°C by *B. subtilis* and *Bacillus circulans*. Immanuel et al. (2006) also recorded maximum endoglucanase activity in *Cellulomonas, Bacillus* and *Micrococcus* sp. at 40°C and neutral pH. In this context, 2b and 38b growth was found at 30°C. The growth of bacterial isolates at room temperature minimizes the expenditure on temperature control at the scale up of the process. This is an advantage on previously reported strains. The bacterial strains 2b and 38b was optimized for the production of cellulase at 30°C. This study will be a milestone in the production of the cellulase from bacterial isolates at room temperature.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abdel-Mawgoud M, Aboulwafa MM, Hassouna NAH (2008). Optimization of Surfactin production by *Bacillus subtilis* isolate BS5. Appl. Biochem. Biotechnol. 150(3):305-325.

Apun K, Jong BC, Salleh MA (2000). Screening and isolation of a cellulolytic andamylolytic *Bacillus* from sago pith waste. J. Gen. Appl Microbiol. 46(5):263-267.

Assareh R, Zahiri HS, Noghabi KA, Aminzadeh S, Khaniki B (2012). Characterization of newly isolated *Geobacillus sp*. T1, the efficient cellulose producer on untreated barley and wheat straws. Bioresour. Technol. 120:99-105.

Bakare MK, Adewale IO, Ajayi A, Shonukan OO (2005). Purification and characterization of cellulose from the wild-type and two improved mutants of *Pseudomonas fluorescens*. Afr. J. Biotechnol. 4(9):898-904.

Bhat MK (2000). Cellulases and related enzymes in biotechnology. Biotechnol. Adv. 18(5):355-383.

Buchanan RE, Gibbons NE (1974). Bergey's of Determinative Bacteriology. Williams & Wilkins Co., Philadelphia, PA, USA.

Callow N, Ray CS, Kelby MA, Ju LK (2016). Nutrient control for stationary phase cellulase production in Trichoderma reesei Rut C-30. Enzyme Microb.Technol. 82:8-14.

Chantawannakul P, Oncharoen A, Klanbut K, Chukeatirote E, Lumyong S (2002). Characterization of cellulases of *Bacillus subtilis* strain 38 isolated from traditionally fermented soybean in northern Thailand. Sci. Asia 28:241-245.

Cirigliano MNF, Rezende RDC, Oliveira MNG, Pereira PHF, Nascimento RPD, Bon EPDS, Macrae A, Coelho RRR (2013). *Streptomyces misionensis* PESB-25 produces a thermoacidophilic endoglucanase using sugarcane bagasse and corn steep liquor as the sole organic substrates. Biomed Res. Int. vol. 2013, Article ID 584207, 9 p.

Immanuel G, Dhanusha R, Prema P, Palavesam A (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. Int. J. Environ. Sci. Technol. 3(1):25-34.

Jansova E, Schwarzová Z, Chaloupka J (1993). Sporulation and synthesis of extracellular proteinases in *Bacillus subtilis* are more temperature-sensitive than growth. Folia Microbiol. 38(1):22-24.

Jarvis M (2002). Cellulose stacks up. Nature 426:611-612.

Lan TQ, Wei D, Yang ST, Liu X (2013). Enhanced cellulase production by *Trichoderma viride* in a rotating fibrous bed bioreactor. Bioresour. Technol. 133:175-182.

Lee YJ, Kim BK, Lee BH, Jo KI, Lee NK, Chung CH, Lee YC, Lee JW (2008). Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL-3 utilizing rice hull. Bioresour. Technol. 99(2):378-386.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31(3):426-428.

Nakamura K, Kppamura K (1982). Isolation and identification of crystalline cellulose hydrolysing bacterium and its enzymatic properties. J. Ferment. Technol. 60(4):343-348.
Rastogi G, Bhalla A, Adhikari A, Bischoff KM, Hughes SR, Christopher LP, Sani RK (2010). Characterization of thermostable cellulases produced by *Bacillus* and *Geobacillus* strains. Bioresour. Technol. 101:8798-8806.
Ray K, Bairagi A, Sarkar Ghosh K, Sen SK (2007). Optimization of fermentation conditions for cellulose production by *Bacillus subtilis* CY5 and *Bacillus circulan* TP3 isolated from fish gut. Acta Ichthyologica et Piscatoria 37(1):47-53.
Saini R, Saini JK, Adsul M, Patel AK, Mathur A, Tuli D, Singhania RR (2015). Enhanced production by *Penicillium oxalicum* for bio-ethanol application. Bioresour. Technol. 188:240-246.
Sarita M, Arora A, Singh S, Nain L (2013). *Streptomyces griseorubens* mediated delignification of paddy straw for improved enzymatic saccharification yields. Bioresour. Technol. 135:12-17.
Sethi S, Datta A, Lal Gupta B, Gupta S (2013). Optimization of cellulase production from bacteria isolated from soil. ISRN Biotechnol. 985685:1-7.
Shanmugapriya K, Saravana PS, Manoharan M, Mythili A, Joseph S (2012). Isolation, screening and partial purification of cellulase from cellulase producing bacteria. Int. J. Adv. Biotechnol. Res. 3:509-514.
Sharma B, Agrawal R, Singhania RR, Satlewal A, Mathur A, Tuli D, Adsul M (2015). Untreated wheat straw: Potential source for diverse cellulolytic enzyme secretion by *Penicillium janthinellum* EMS-UV-8 mutant. Bioresour. Technol. 196:518-524.
Zhang Y-HP, Lynd LR (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnol. Bioeng. 88:797-824.