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Isolation and characterization of cellulolytic *Bacillus licheniformis* from compost

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Eight cellulose degrading bacteria were isolated from compost and were identified as *Bacillus licheniformis* by 16S rRNA sequencing. Among the eight isolates, *Bacillus licheniformis* B4, B7 and B8 showed the highest cellulase activity. *B. licheniformis* B4 and B8 showed the maximum cellulase activity during the stationary phase of growth; but for B7, the maximum activity of cellulase was observed during the log phase. Reducing sugar released in the media, increased with increasing cellulase activity for all the three isolates. Significant correlation was observed between cellulase activity and protein content. The crude cellulase from B7 strain showed activity towards carboxymethyl cellulose and filter paper, but there was no detectable activity towards *p*-nitrophenyl- β-D-glucopyranoside (PNPG). The crude cellulase of *B. licheniformis* B7 exhibited maximum activity at 50°C and at pH 6 to 7.

Key words: *Bacillus licheniformis*, 16S rRNA, cellulase, reducing sugar, compost, viscosity.

INTRODUCTION

Accumulation of municipal solid waste is becoming a serious problem in all developing countries (Al-Khatib et al., 2015). In most developing countries, the inadequate treatment of municipal solid waste causes a serious threat to the environment (Pin-Jing, 2012). Cellulosic wastes such as paper, wood, agricultural residues and cardboard constitute a major component of municipal solid waste. Improper management of these wastes contaminates air, soil and water. Disposal of solid wastes in landfills contaminates the ground water and cause the emission of greenhouse gases such as carbon dioxide and methane. Most of the carbon dioxide and methane are produced from biodegradable cellulosic wastes such as wood, leaves, other agricultural residues and waste papers. Therefore, recycling of such cellulosic wastes can decrease the greenhouse effect (Kazaragis, 2005) and these can be used as one of the main renewable sources of energy (Zhou et al., 2015).

Cellulose a linear polysaccharide composed of β-1,4 linked D-glucopyranosyl units is synthesized by all higher

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plants and other organisms such as bacteria, fungi, protists and invertebrates. It is considered as the most abundant renewable natural biological resource on the earth (Zhang et al., 2006). There is an increasing interest in the production of biofuel using cellulosic biomass as a renewable source of energy by breaking them into sugars using cellulase enzymes (Demain et al., 2005). The production of fuels and chemicals from cellulosic substrates using cellulases would reduce the use of fossil fuels and decrease the air pollution (Zhou et al., 2001). In biorefining, renewable resources such as agricultural crops or wood are utilized either for extraction of intermediates or for bioconversion into chemicals, commodities and fuels (Kamm and Kamm, 2004; Fernando et al., 2006). Thermostable enzymes has an advantage as catalysts in these processes, as high temperatures often promote better enzyme diffusion and cell-wall disorganization of the raw materials (Paes and O'Donohue, 2006). Furthermore, the conversion of plant-derived cellulosic biomass into useful commodities as biofuels always depends on the cellulase producing efficiency of bacteria.

Cellulases have extensive applications in various industries. Traditionally, they are useful in food and brewery production, animal feed processing, detergent production and laundry, textile processing and paper pulp manufacturing (Kuhad et al., 2011; Karmakar and Ray, 2011). The applications of cellulase enzymes in cellulose biorefinery for producing fermentable sugars are expected to rapidly increase in the foreseeable future due to the problems in sustainable supply of fossil fuel and the increased demand for production of biofuels and chemicals from renewable resources (Juturu and Wu, 2014). Because of the booming biotechnology industries, the demand for thermostable enzymes has increased immensely due to its high thermostability and feasibility (Haki and Rakshit, 2003).

Reactions at higher temperatures has decreased viscosity and hence increased diffusion coefficient of substrates leading to the favourable equilibrium displacement in endothermic reactions (Kumar and Swati, 2001). Conducting biotechnological processes at high temperatures reduce the risk of contamination by common mesophiles. In addition, process at high temperatures has major impact on the bioavailability and solubility of organic compounds leading to the effective bioremediation (Becker, 1997). Thus the demand for cellulase producing bacteria is steadily increasing, and the search of cellulase degrading bacteria from different sources is continuously needed.

This study was conducted with an objective to isolate cellulase producing bacteria from compost, an important component in the organic farming. The organic matter in compost is mainly cellulose (Jurak et al., 2014) and hence compost can be a good source to isolate cellulase producing bacteria.

MATERIALS AND METHODS

Screening of cellulolytic bacteria from compost samples

Twenty five compost samples were collected from the Gulf mushroom company Muscat, Oman for isolation of cellulase producing bacteria. The temperature at the time of collection was 50°C, and the samples were brought to the laboratory immediately after collection and well assay was used to screen the cellulose degrading capacity of bacteria from different compost samples. Five wells were made in a sterile carboxymethyl cellulose (CMC) medium (1 g of CMC, 0.1 g of NaCl, 0.1 g of $NaNO_3$, 0.1g of K_2HPO_4, 0.1g of KCl, 0.05 g of $MgSO_4$, 0.05 g of yeast extract, 1.7 g of agar and 100 ml of distilled water).

Compost samples (0.5 g) were dissolved in 4.5 ml Ringer solution (1/4 strength – Sigma). After homogeneous mixing, 100 µl of samples was added to each well. The plates were incubated at 50°C for 24 h. After incubation the CMC plates were flooded with Congo red, incubated for 30 min and then destained with 1 M NaCl. The compost samples with large clearance zones were selected for the isolation of cellulase producing bacteria. To isolate cellulolytic bacteria, 1 g of the selected compost samples were serially diluted using an automatic spiral platter (Autoplate 4000, Spiral Biotech, and UK), 100 µl of the sample was plated on the CMC agar medium and incubated at 50°C for 24 h. Colonies of cellulolytic bacteria were counted and the clearing zone diameters of the colonies were measured in mm (Apun et al., 2000).

Molecular identification of the isolates

Among the cellulase producing bacteria, eight isolates with the largest diameter of clearing zones were selected, isolated and identified by 16S rRNA sequence. Bacterial DNA was extracted and purified (MoBio kit, USA). The purified DNA was amplified by 30 cycles of PCR in a thermal cycler (Applied Biosystem, USA). Two external primers annealing at 5 and 3' end of the 16S rRNA were used: B27f 5'-AGAGTTTGATCCTGGCTCAG-3' and U1492r 5'-GGTTACCTTGTTACGACTT-3'. The amplified products were analyzed in 1% agarose gel and purified (Aquick PCR purification kit, UK). The purified products were subjected to a second PCR and the products were sequenced using 3130X1 Genetic Analyzer (Applied Biosystem, UK). In the second PCR, two internal primers A341f 5'-CCTAXGGGGXGCAXCAG-3' and A1041r 5'-GGCCATGCACCWCCTCTC-3'were used at 5 and 3' ends of 16S rRNA. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Utilization of substrates

A preliminary study was conducted to demonstrate the ability of the eight isolates in utilizing glucose, cellobiose, mannose, D-xylose, L-xylose, D-arabinose, L-arabinose and methyl-β-D-xylopyranose in phenol broth. Bacteria were inoculated into the broth aseptically and incubated at 50°C for 24 h. Change of the phenol broth from red to yellow is an indication of utilization of the above sugars.

Inoculum preparation and cellulase fermentation

For inoculum preparation, the eight isolates were cultured in

nutrient broth at 50°C. Once OD reached 0.5 (at 600 nm), it will be use as inoculum for the production media. Inoculum of each bacteria (3%) was inoculated into 50 ml in two production media containing 1 and 2% CMC in 250 ml Erlenmeyer flasks and incubated at 50°C in a rotary shaker at 100 rpm. The supernatants which was obtained after centrifugation at 10,000 rpm (Beckman Coulter) at 24, 48 and 72 h were measured for cellulase activity.

Cellulase activity

The cellulase activity was estimated using 2% CMC in 0.05 M citrate buffer (pH 4.8) as a substrate. Substrate (0.5 ml) was added to culture filtrate (0.5 ml) and incubated for 30 min at 50°C. Reducing sugar released was estimated by dinitrosalicylic acid (DNS) method (Ghose, 1987). The cellulase activity was determined by using a calibration curve of glucose. One unit of CMCase activity was expressed as 1 µmol of glucose which was liberated per ml enzyme per minute.

Cellulase production

To find out the cellulase producing ability, the selected isolates were inoculated into the CMC broth at 50°C. During fermentation, samples was withdrawn for every 2 h time interval and analysed for cellulase activity, reducing sugar, cell growth, viscosity, pH and protein content (Lowry et al., 1951).

Effect of temperature and pH on the activity of cellulase

The optimum temperature for CMCase activity of the crude cellulase was determined by measuring the enzyme activity at different temperatures ranging from 30 to 90°C. The optimum pH was also determined by measuring the enzyme activity at different pH ranging from 3 to 11 at optimum temperature. Different buffers such as 50 mM sodium acetate buffer (pH 3, 4 and 5), 50 mM sodium phosphate buffer (pH 6, 7, and 8) and 50 mM glycine-NaOH buffer (pH 9, 10 and 11) were used to adjust the pH. Further, crude cellulase was also tested for its ability to degrade CMC, filter paper, cellobiose, *p*-Nitrophenyl β-D-glucopyranoside (PNPG) and methyl-βD-xylopyranose.

Statistical analysis

The mean values and standard deviations were calculated form the data obtained from three different experiments. Analysis of variance was performed by the one way ANOVA procedures followed by Tukey HSD Post Hoc tests using PASW statistics 8. Statistical difference at $p< 0.05$ was considered significant.

RESULTS AND DISCUSSION

In this study, compost was selected as a source for the isolation of cellulase producing bacteria, because the organic content in compost is mainly made up of cellulose and the temperature during composting was 50°C and above. Cellulase producing microbes have been isolated from different sources such as soil, water, compost etc.(Al-Kharousi et al., 2015). All the compost samples were positive for cellulolytic bacteria by forming clear zones in different diameters ranging from eight to nineteen mm. However, only 11 out of 25 samples showed that clearing zones is equal or greater than 10 mm. These samples were selected for isolation of cellulase producing bacteria. The colony forming units (CFU) of these samples ranged from $20x10^3$ to $82x10^3$ CFU/ml. Totally 25 bacteria were isolated from the 11 samples. Out of which, eight isolates with a large diameter of the clear zone were selected for further studies. The nucleotide sequencing of 16S rRNA confirmed that all the eight isolates were different strains of *Bacillus licheniformis* and named as *B. licheniformis* B1 to B8 (NCBI accession no JQ700446 to JQ700453).

Amplified 16S ribosomal ribonucleic acid (rRNA) gene fragments from the isolated *B. licheniformis* strains were sequenced and blast searched through the The National Center for Biotechnology Information (NCBI) database. Closely related sequences were downloaded and aligned using CLUSTAL W. These sequences were analyzed using the maximum likelihood method. *Acetobacter pasteurianus* 386B with accession number 102925.1 was taken as an out group (Figure 1). The presence of only *B. licheniformis* could be due to the high temperature of compost which allows only the endospore formers to exist. *Bacillus* spp. are the potential producers of cellulases. Other investigators reported that moderately thermophilic *B. licheniformis* was found to hydrolyze carboxymethylcellulose and *p*-nitrophenylcellobioside (Bischoff et al., 2006). *B. licheniformis* with high cellulase activity at 65°C was isolated from mangroves (Tabao and Monsalud, 2010). *B. licheniformis* was able to degrade rice straw by secretion of cellulase and hemicellulase (Hong et al., 2007). *B. amyloliquefaciens* hydrolyzing rice hull was isolated from soil (Lee et al., 2008). Most celluloytic *Bacillus* spp. secrete endoglucanases which are capable of degrading carboxymethyl cellulose (Robson and Chambliss, 1984). Mostly *Bacillus* spp. are used in industries because they are not pathogenic, grow and reproduce easily, do not produce foul odors or gases, some species can survive in alkaline condition and at high temperature, secrete proteins extracellularly and are considered relatively safe to use with regard to health and environmental aspects (Beukes and Pletschke, 2006).

Utilization of different substrates

The ability of the eight isolates to utilize different substrates and derivatives of cellulose as carbon sources were studied. All the isolates were able to utilize xylan, cellobiose, glucose and mannose. None of the isolates utilized methyl-β-D-xylopyranose, L-xylose and D-arabinose. However, D-xylose was utilized only by *B. licheniformis* strains B4, B7 and B8 and L-arabinose by B4 and B7 (Table 1). Both xylose and arabinose are the

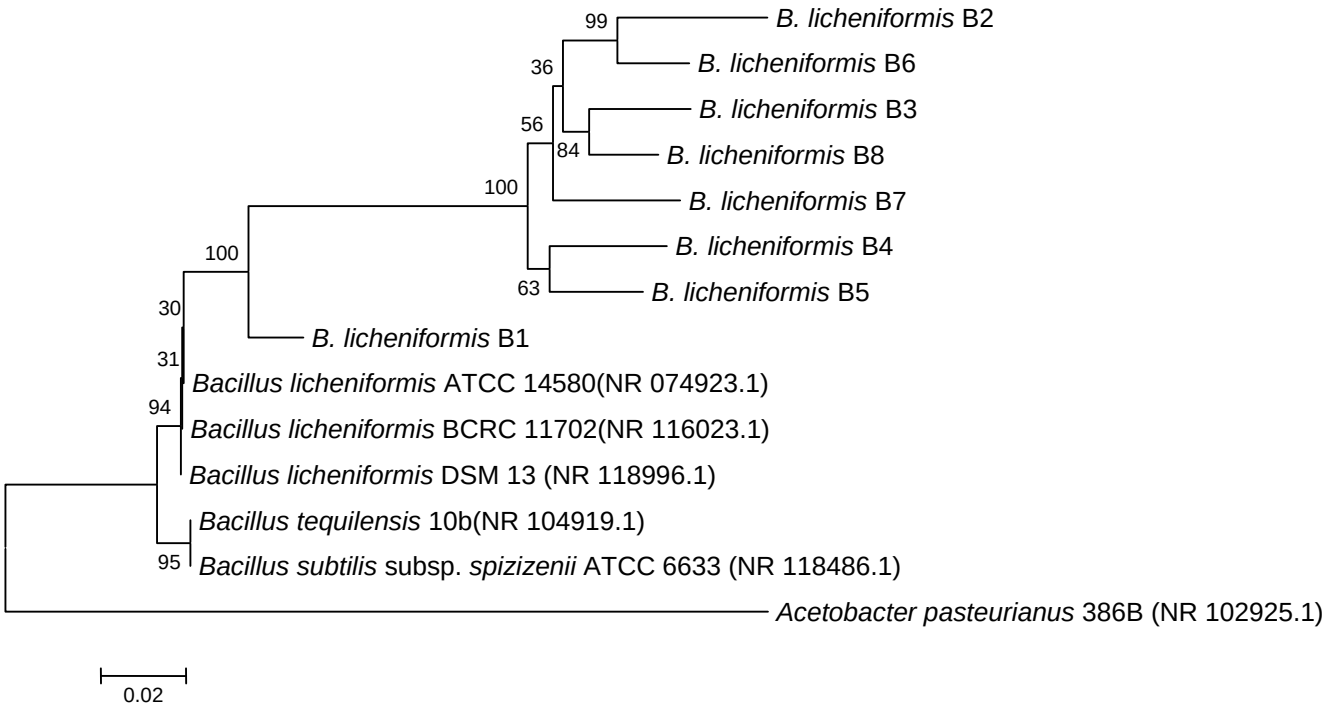

Figure 1. Phylogenetic tree of *B. licheniformis* spp. isolated from compost.

building blocks of hemicellulose.

This indicates that all the eight bacteria isolated from compost have the ability to secrete xylanase, cellobiase, mannase and CMCase. Hydrolysis of complex plant cellulosic materials require the presence of different enzymes to degrade cellulose and hemicellulose into simple sugars. Enzymes, such as CMCase, xylanase, cellobiohydrolases, β-glucosidases, endo-1,4-β-xylanases, β-xylosidases, α-l-arabinofuranosidases, acetyl xylan esterase, α-glucuronidase, pectate lyase, and endo-β-1,4-d-mannanase are needed in biodegradation of cellulosic biomass (Van Dyk et al., 2009). This suggests that the isolates of this study were able to degrade the cellulose.

Influence of CMC on cellulase production

Cellulases are inducible enzymes produced by microorganisms when grown on cellulose and cellulose derivatives. The influence of CMC concentration on cellulase production and microbial density was assessed for the eight isolates using 1 and 2% CMC media. In 1% CMC medium, B3 showed the high cell density while B5 and B7 showed the lowest cell density (Figure 2a). The changes in cell densities during 24 h (p=0.089) and 48 h (p=0.103) were insignificant for all the isolates. However, cell density of B5 decreased significantly at 72 h and B3 increased significantly at 72 h (p=0.03). In 1% CMC medium, B7 showed the maximum cellulase activity at 48 h (Figure 2b). On the other hand, B3, B5 and B6 showed high cellulase activity at 72 h. All the eight isolates didn't show any significant difference in their cellulase activity at 24 h (p>0.05). However, significant differences were found in the cellulase activity among all the eight isolates during 48 and 72 h (p=0.047 and 0.049 respectively).

In 2% CMC medium, cell densities of the isolates varied significantly from each other (p>0.05). For all the isolates microbial density declined during 72 h (Figure 3a). B2 showed lower cell density but B6 exhibits high cell density among the isolates. High cellulase activity was observed in B7 at 24 h. Among the isolates, cellulase activity was low in B5 and B6 even though they showed a good growth in the medium (Figure 3b). The induction of enzyme activity is significantly higher in 2% CMC medium than 1% medium. CMC is the most effective carbon source for cellulase production by *B. alcalophilus* and *B. amyloliquefaciens* (Abou-Taleb et al., 2009). *B. pumilus* EB3 was induced to produce CMCase when grown in the CMC medium (Ariffin et al., 2006). In this study, it is interesting to note that some isolates showed high cell density in the medium but their cellulase activity is still low. Some other strains with moderate cell density showed high cellulase activity in both 1% and 2% CMC media suggesting that the bacteria with high cell density may not be a high cellulose producer (Tong and

Table 1. Utilization of different carbon sources by *B. licheniformis* strains isolated from compost samples.

Strains with NCBI accession number	xylan	Cellobiose	methyl-βD-xylopyranose	D-xylose	L-xylose	D-arabinose	L-arabinose	Glucose	Mannose
B. licheniformis B1 (JQ700446)	+	+	-	-	-	-	-	+	+
B. licheniformis B2 (JQ700447)	+	+	-	-	-	-	-	+	+
B. licheniformis B3 (JQ700448)	+	+	-	-	-	-	-	+	+
B. licheniformis B4 (JQ700449)	+	+	-	+	-	-	+	+	+
B. licheniformis B5 (JQ700450)	+	+	-	-	-	-	-	+	+
B. licheniformis B6 (JQ700451)	+	+	-	-	-	-	-	+	+
B. licheniformis B7 (JQ700452)	+	+	-	+	-	-	+	+	+
B. licheniformis B8 (JQ700453)	+	+	-	+	-	-	-	+	+

+ able to utilize carbon source; - unable to utilize carbon source.

Rajendra 1992; Emtiazi et al., 2007). Components in the media, other than CMC, might influence the growth to a certain extent. Among the eight isolates, the best three (B4, B7 and B8) were selected for further studies after enrichment in CMC medium.

Relationship between cell growth and cellulase activity

Both B4 and B8 had a high positive significant correlation between cellulase activity and pattern of cell growth (Figure 4a and c). For B4 and B8 strains, the cellulase activity reached a maximum between 14 and 18 h when the growth is at the stationary phase. This could be due to the decreasing concentration of CMC in the medium which results in the slowing down of growth rate and the cell density maintained at the same level. Hence, the cellulase production does not increase after this level (Robson and Chambliss 1984) and the enzyme activity follows the cell growth pattern. A positive significant correlation between cellulase activity and cell growth was observed (p<0.05). For B7, maximum cellulase activity occurred after 4 h, in the log phase of growth. This clearly indicates that these bacteria utilize CMC as a source of carbon and energy by producing cellulase as a primary metabolite. Further, enrichment in CMC medium increased the cellulase activity of B7. Cellulase activity and growth of B7 strain were not significantly correlated (Figure 4b). The time to reach the maximum cellulase activity for B4, B7 and B8 could be due to the difference in the triggering of cellulase pathways by cellulose. This also indicates that the correlation between cell growth and cellulase activity depends on the bacterial strain.

Among the isolates B4, B7 and B8, the B7 has a novel feature of producing maximum cellulase activity at 4h of incubation. Time required to produce maximum cellulase activity varied among bacteria. Maximum cellulase activity was observed for *Anoxybacillus flavithermus* EHP2 at 36 to 48 h (Ibrahim and El-diwany, 2007), 24 h for *B. subtilis* KO (Shabeb et al., 2010), after 12 h for *B. licheniformis* and after 10 h of fermentation for *B. subtilis* (Hong et al., 2007). Although several studies reported that cellulase activity is higher than the isolates of this study, none of the reports showed maximum cellulase activity of *Bacillus* spp. after 4 h incubation. This variation could be due to the differences in genetic make-up for different strains, and the cultural conditions employed during the production process (Bajaj et al., 2009). Further, the arid environment in Oman from which the organic matters is collected for composting would influence the metabolic activities significantly. A further complete optimization study is required to get increased cellulase production from these strains.

Changes in reducing sugar, protein content, viscosity and pH

Changes in reducing sugar content, protein content, viscosity and pH of the CMC medium were studied. Reducing sugar released into the media indicates the biodegradation of CMC by cellulase enzyme into simple sugar (Alam et al., 2005). The reducing sugar released by all the three isolates followed the trend of cellulase activity (Figure 5a, b and c). A significant positive correlation was observed between cellulase

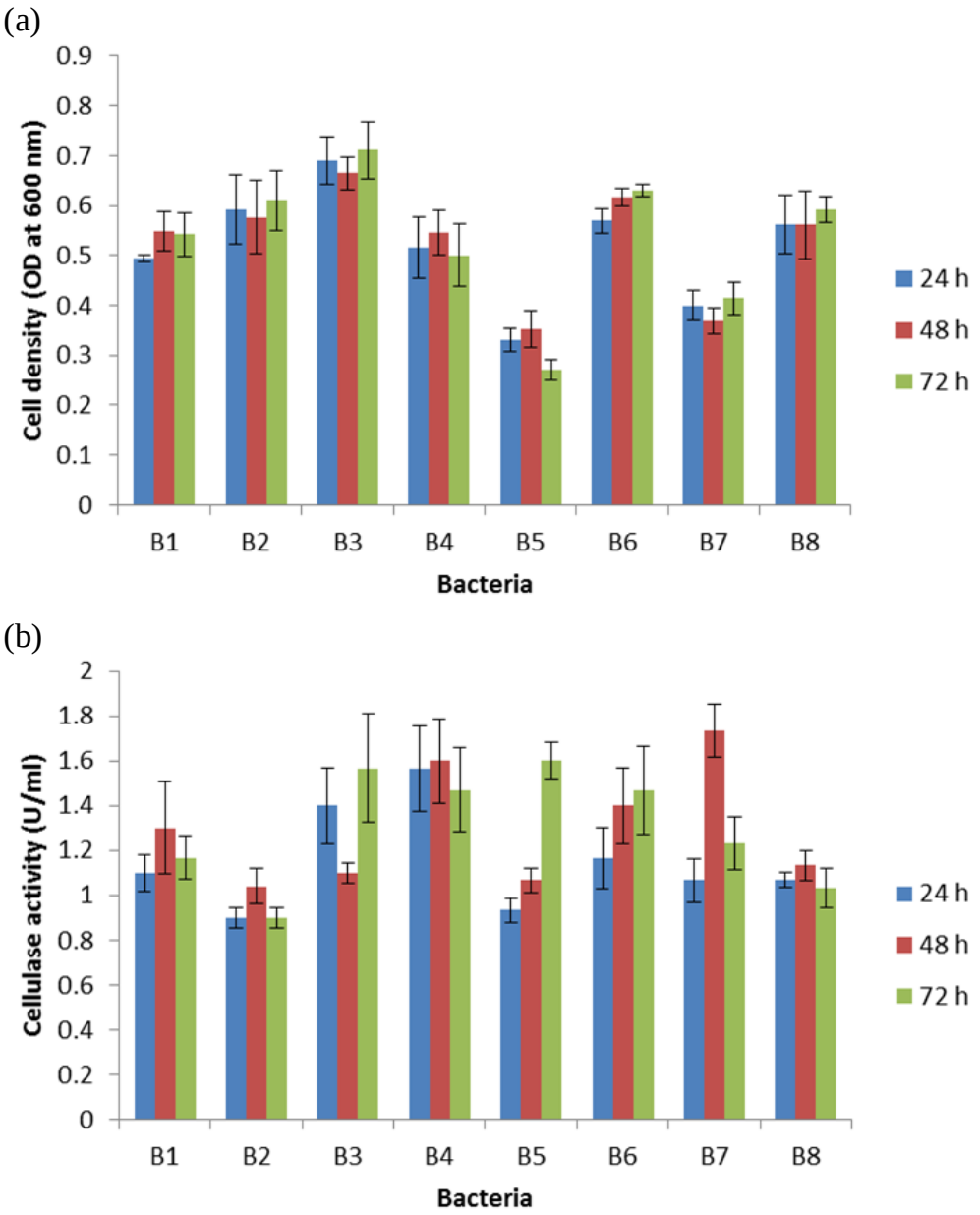

Figure 2. (a) Cell density and (b) cellulase activity of *B. licheniformis* B1–B8 in 1% CMC medium at different incubation time.

activity and reducing sugar released (p<0.05). Cellulase activity is related to soluble enzymes secreted into the medium and therefore the concentration of soluble proteins was analyzed. The increase in cellulase activity was significantly correlated with the increase in soluble protein in the media (p=0.000 for B8 and p<0.05 for B4 and B7).

During cellulase fermentation, the viscosity of B4 inoculated CMC medium decreased up to 8 h and then increased slightly. The viscosity of the B7 inoculated medium dropped sharply at 2 h of incubation and remained constant at later stages of incubation. In B8 inoculated into CMC medium, the viscosity decreased continuously up to 10 h, then increased slightly and remained at the same level during the later stages of incubation (Figure 6 a, b and c). The drop in the viscosity of the CMC medium during the initial hours of fermentation was due to the degradation of CMC by cellulase secreted by the bacteria. The slight increase in viscosity during the later stages of fermentation could be

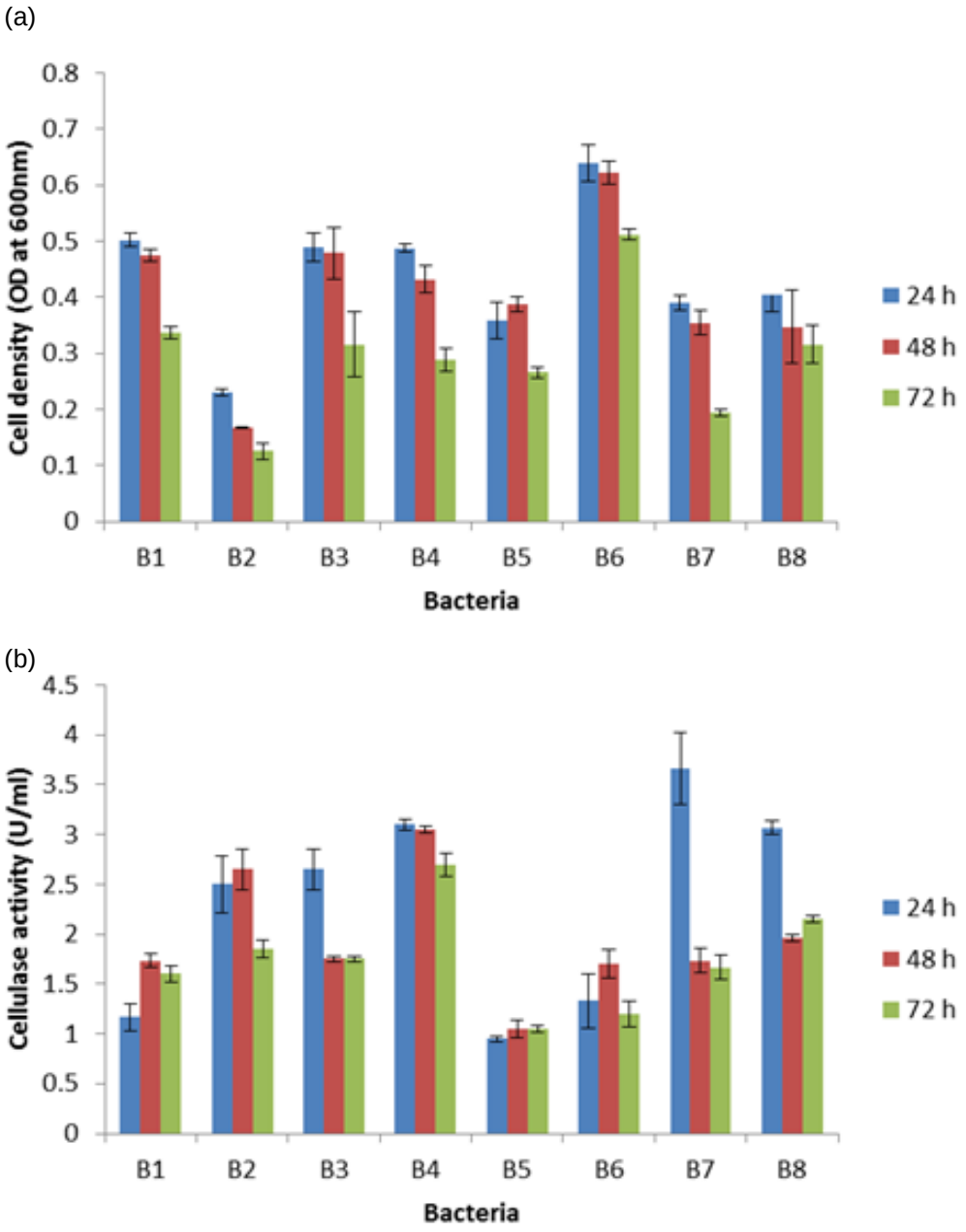

Figure 3. (a) Cell density and (b) cellulase activity of *B. licheniformis* B1 – B8 in 2% CMC medium at different incubation time.

due to the production of exopolysaccharides by the three isolates (Dupont et al., 2000). For all the three bacteria, pH changed from 8.4 to around 9 during cellulase fermentation.

Activity of cellulase at different temperature and pH

During cellulase fermentation B7 showed more activity in a short time than B4 and B8. Hence, the crude cellulase of B7 was tested at different pH and temperature to determine the optimum conditions of activity. The crude cellulase activity was higher at pH 6 and 7 and it retains 80% activity from pH 3 to 10 (Figure 7a). The activity of cellulase in a wide range of pH indicates that this enzyme could be used in different industrial processes with different pH ranges. Cellulases produced by *Bacillus* spp. from different sources have been found to have a different optimum pH.

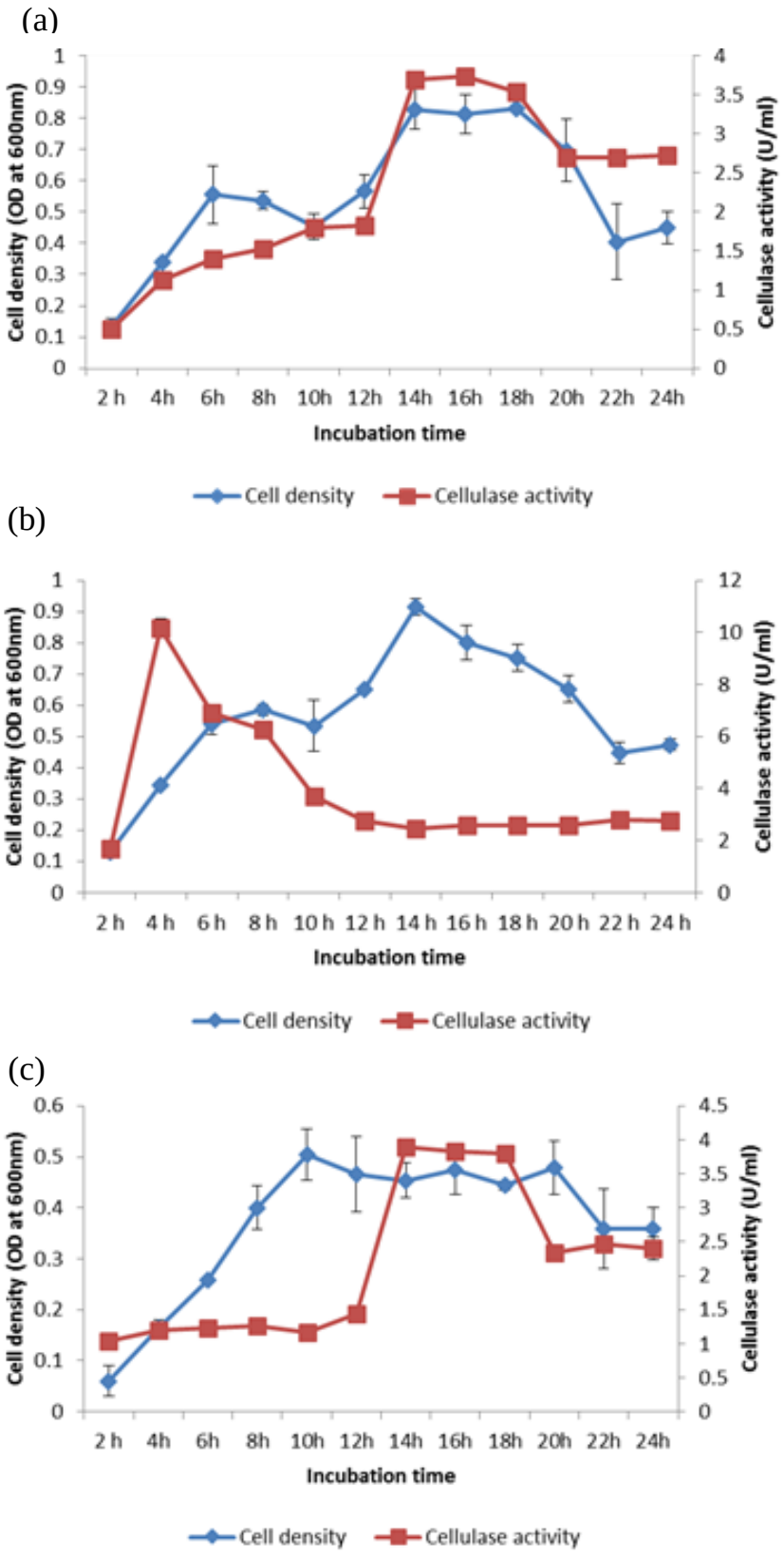

Figure 4. Changes in cell density and cellulase activity of *B. licheniformis:* (a) B4, (b) B7 and (c) B8 during cellulase fermentation in CMC broth.

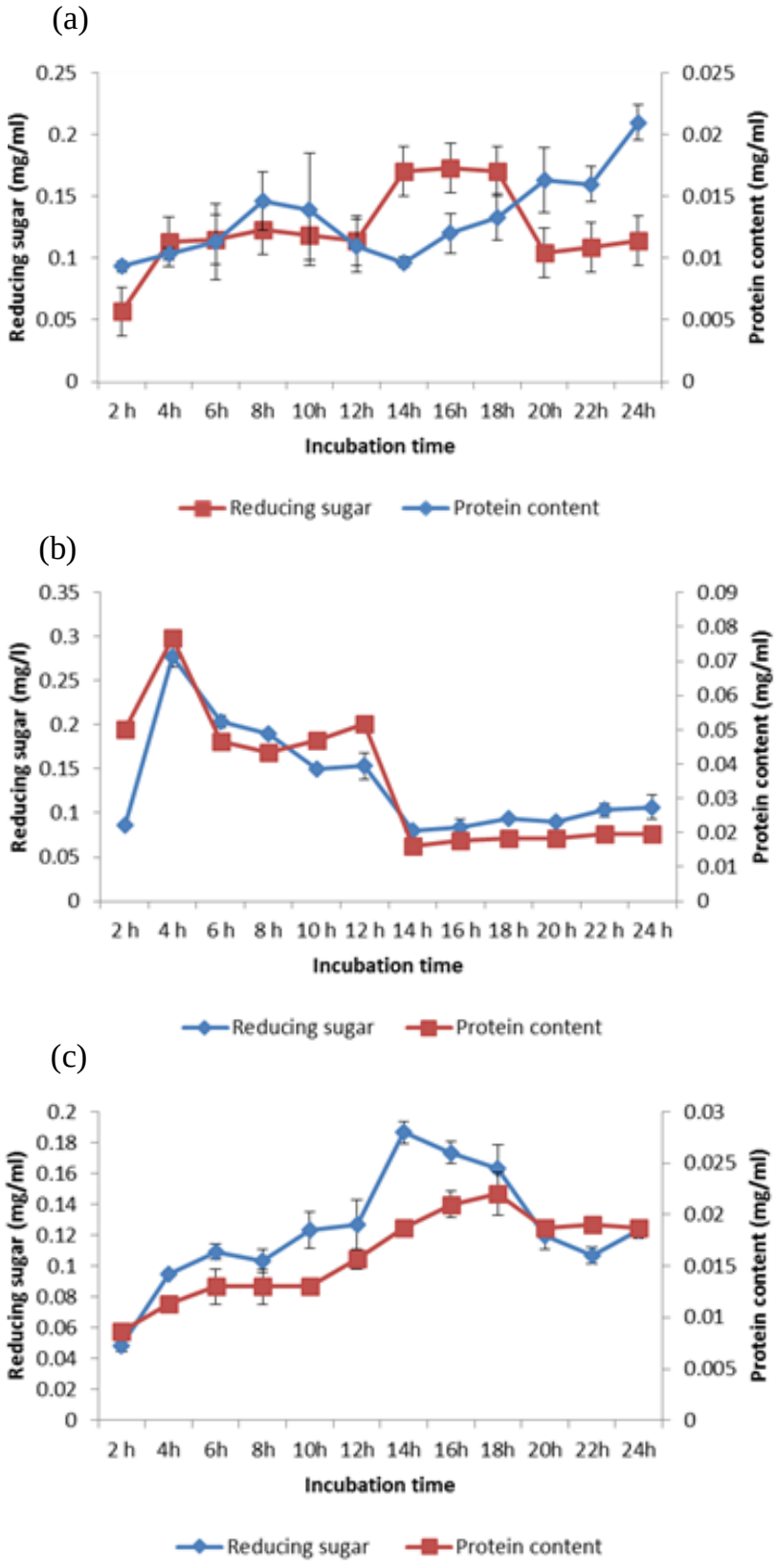

Figure 5. Reducing sugar released and protein content of CMC medium during fermentation by *B. licheniformis.* **(a)** B4, **(b)** B7 and **(c)** B8.

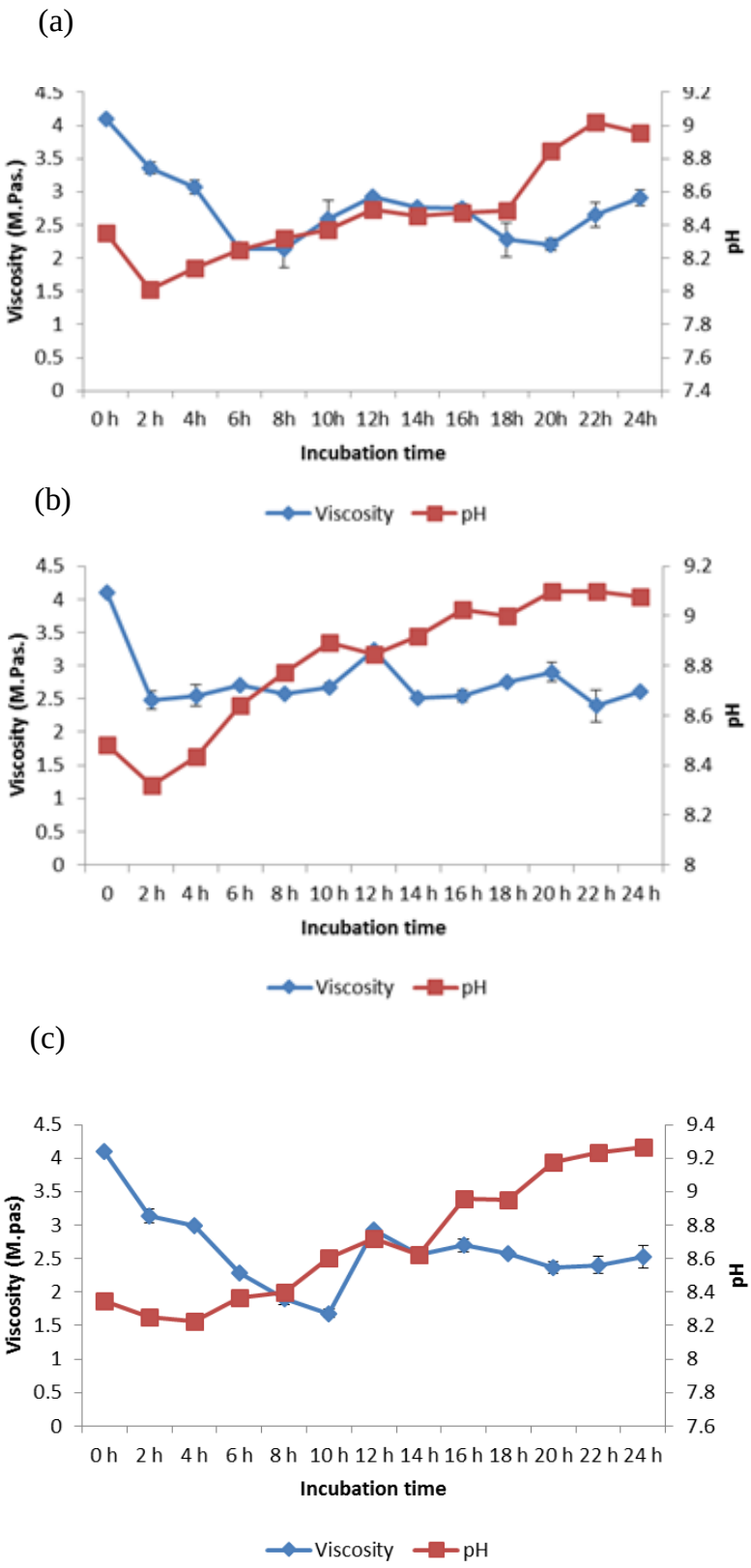

Figure 6. Changes in medium viscosity and cellulase pH of *B. licheniformis* (a) B4, (b) B7 and (c) B8 during cellulase fermentation.

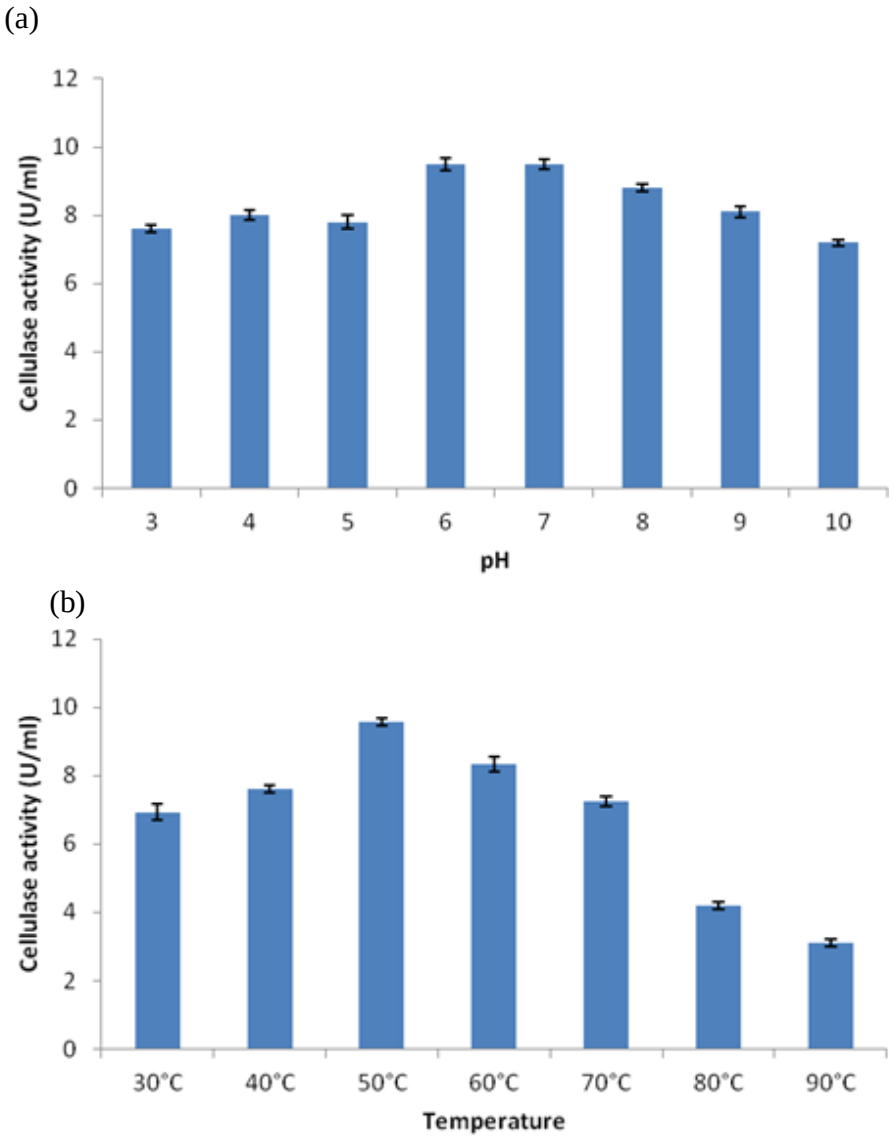

Figure 7. Activity of crude cellulase of *B. licheniformis* B7 at (a) different pH, (b) different temperature.

The optimum pH for *Bacillus* spp. CH43 and HR68 ranges from 5 to 6.5 (Mawadza et al., 2000), and pH 7.5 for *B. licheniformis* NLRI-X33 (Tae-IK et al., 2000). However, other studies have reported that the optimum pH for purified cellulase from *B. circulans* was 4.5 (Kim, 1995) while cellulase produced by *Bacillus* sp. C14 was 11 (Aygan and Arikan 2008). The crude cellulase activity was tested at different temperatures. The optimum temperature for cellulase activity was 50°C (Figure 7b). At 30°C it retains 73% activity and 75% activity at 70°C. The cellulase activity decreased significantly above 70°C and retains only 32% activity at 90°C. It has been reported that the optimum temperature for purified cellulase activity of *Bacillus* strains was 50°C (Kazaragis 2005; Lee et al., 2008; Kim 1995) and for *Anoxybacillus flavithermus* was 75°C (Shabeb et al., 2010). Further, crude cellulase from B7 showed the ability to degrade CMC, filter paper (FPase activity of 0.035 U/mg) and cellobiose (cellobiase activity of 0.04 U/mg). However, there was no detectable activity towards PNPG and methyl-βD-xylopyranose.

Conclusions

Eight cellulolytic *B. licheniformis* strains have been isolated from compost samples which were able to utilize

xylan, cellobiose, mannose and CMC. Among the eight isolates, B4, B7 and B8 were found to produce the highest cellulase activity. B7 strain was found to produce the maximum cellulase activity at 4 h of incubation.

Moreover, the crude cellulase from B7 was active in a wide range of pH with an optimum temperature of 50°C at pH 6 to 7. Because of the ability to retain good activity at higher temperatures and a wide range of pH, cellulase from *B. licheniformis* B7 could be useful in biorefineries. An optimization study would further increase the production of cellulase by these bacteria. Further, the compost would be a good resource to isolate cellulose degrading bacteria.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Abou-Taleb KAA, Mashhoor WA, Nasr SA, Sharaf MS, Abdel-Azeem HHM (2009). Nutritional and Environmental Factors Affecting Cellulase Production by Two Strains of Cellulolytic Bacilli. Aust. J. Basic Appl. Sci. 3:2429-2436.

Alam MZ, Nurdina M, Erman MM (2005). Production of cellulase from oil palm biomass as substrate by solid state bioconversion. Am. J. Appl. Sci. 2:569-372.

Al-Khatib IA, Kontogianni S, Nabaa HA, Alshami N, Al-Sari MI (2015). Public perception of hazardousness caused by current trends of municipal solid waste management. Waste Manag. 36:323-330.

Al-Kharousi MM, Sivakumar N, Elshafie A (2015). Characterization of cellulase enzyme produced by *Chaetomium* sp. isolated from books and archives. Eurasia. J. Biosci. 9:52-60.

Apun K, Jong BC, Salleh M (2000). Screening and isolation of a cellulolytic and amylolytic *Bacillus* from sago pith waste. J. Gen. Appl. Microbiol. 5:263-67.

Ariffin H, Abdullah MS, Kalsom U, Shirai Y, Hassan MA (2006). Production and characterization of cellulase by *Bacillus pumilus* EB3. Int. J. Eng. Technol. 3:47-53.

Aygan A, Arikan B (2008). A new halo-alkaliphilic, thermostable endoglucanase from moderately halophilic *Bacillus* sp. C14 isolated from Van Soda Lake. Int. J. Agric. Biol. 10:369-374.

Bajaj BK, Himani P, Wani WMA, Priyanka S, Ajay S (2009). Partial purification and characterization of a highly thermostable and pH stable endoglucanase from a newly isolated *Bacillus* strain M-9. Indian J. Chem. Techn. 16:382-387.

Becker P (1997). Determination of the kinetic parameters during continuous cultivation of the lipase producing thermophile *Bacillus* sp. IHI-91 on olive oil. Appl. Microbiol. Biotechnol. 48:184-190.

Beukes N, Pletschke BI (2006). Effect of sulfur-containing compounds on *Bacillus* cellulosome associated CMCase and Avicelase activities. FEMS Microbiol. Lett. 264: 226-231.

Bischoff KM, Rooney AP, Li XL, Liu S, Hughes SR (2006) Purification and characterization of a family 5 endoglucanase from a moderately thermophilic strain of *Bacillus licheniformis*. Biotechnol. Lett. 28:1761-1765.

Demain AL, Newcomb M, David Wu JH (2005). Cellulase, clostridia, and ethanol. Microbiol. Mol. Biol. Rev. 69:124-154.

Dupont I, Roy D, Lapointe G (2000). Comparison of exopolysaccharide production by strains of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* grown in chemically defined medium and milk. J. Ind. Microbiol. Biot. 24:251-255.

Emtiazi G, Pooyan M, Shamalnasab M (2007). Cellulase activities in nitrogen fixing *Paenibacillus* isolated from soil in N-free media. World J. Agri. Sci. 3:602-608.

Fernando S, Adhikari S, Chandrapal C, Murali N (2006) Biorefineries: Current status, challenges and future direction. Energy Fuels. 20:1727-1737.

Ghose TK (1987). Measurement of Cellulase Activities. Pure Appl. Chem. 59:257-268.

Haki GD, Rakshit SK (2003). Developments in industrially important thermostable enzymes:a review. Bioresour. Technol. 89:17-34.

Hong Y, Qian Y, Zhong-cheng P (2007). Study on degradation of rice straw by *Bacillus licheniformis*. J Zhejiang Univ. Sci. B. 33:360-366.

Ibrahim ASS, El-diwany AI (2007) Isolation and identification of new cellulases producing thermophilic bacteria from an egyptian hot spring and some properties of the crude enzyme. Aust. J. Basic Appl. Sci. 4:473-478.

Jurak E, Kabel MA, Gruppen H (2014). Carbohydrate composition of compost during composting and mycelium growth of *Agaricus bisporus*. Carbohydr. Polym. 101:281-288.

Juturu V, Wu JC (2014). Microbial cellulases: Engineering, production and applications. Renew Sust. Energ. Rev. 33:188-203.

Kamm B, Kamm M (2004). Principles of biorefineries. Appl. Microbiol. Biotechnol. 64:137-145.

Karmakar M, Ray RR (2011). Current trends in research and application of microbial cellulases. Res. J. Microbiol. 6:41-53.

Kazaragis A (2005). Minimization of atmosphere pollution by utilization of cellulose waste. J. Environ. Eng. Landsci. 2:81-90.

Kim CH (1995). Characterization and substrate specificity of an endo-beta-1,4-D- glucanase I (Avicelase I) from an extracellular multienzyme complex of *Bacillus circulans*. Appl. Environ. Microbiol. 61:959-965.

Kuhad RC, Gupta R, Singh A (2011). Microbial cellulases and their industrial applications. Enzyme Res.pp. 1-10.

Kumar HD, Swati S (2001). Modern Concepts of Microbiology, Second revised edition. Vikas Publishing House Private Limited, New Delhi.

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. 99:378-386.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J. Boil. Chem. 193:265-275.

Mawadza C, Hatti-Kaul R, Zvauya R, Mattiasson B (2000). Purification and characterization of cellulases produced by two *Bacillus* strains. J. Biotechnol. 83:177-187.

Paes G, O'Donohue MJ (2006). Engineering increased thermostability in the thermostable GH-11 xylanase from *Thermobacillus xylanilyticus*. J. Biotechnol. 125:338-350.

Pin-Jing H (2012). Municipal solid waste in rural areas of developing country: do we need special treatment mode? Waste Manag. 32:1289-1290.

Robson LM, Chambliss GH (1984). Characterization of the celluloytic activity of *Bacillus* isolate. Appl. Environ. Microb. 47:1039-1046

Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Shabeb MSA, Magai, Younis AM, Hezayen FF, Nour-Eldein MA (2010). Production of cellulase in low-cost medium by *Bacillus subtilis* KO strain. World Appl. Sci. J. 8:35-42.

Tabao NS, Monsalud RG (2010). Screening and optimization of cellulase production of *Bacillus* strains isolated from Philippine mangroves. Phill. J. Syst. Biol. 5:79-87.

Tae-IK, Han JD, Jeon BS, Yang CB, Kim KN, Kim MK (2000). Isolation from cattle manure and characterisation of *Bacillus licheniformis*

NLRI-X33 secreting cellulase. Asian Austral. J. Anim. 13:427-31.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30: 2725-2729.
Tong CC, Rajendra K (1992). Effect of carbon and nitrogen sources on the growth and production of cellulase enzymes of a newly isolated *Aspergillus sp.* Pertanika 15:45-50.
Van Dyk JS, Sakkab M, Sakkab K, Pletschkea BI (2009). The cellulolytic and hemi-cellulolytic system of *Bacillus licheniformis* SVD1 and the evidence for production of a large multi-enzyme complex. Enzyme Microb. Technol. 45:372-378.
.
Zhang YHP, Himmel ME, Mielenz JR (2006). Outlook of cellulase improvement: screening and selection strategies. Biotechnol. Adv. 24:452-481.
Zhou H, Long YQ, Meng AH, Li QH, Zhang YG (2015). Classification of municipal solid waste components for thermal conversion in waste-to-energy research. Fuel 145:151-157.
Zhou S, Davis FC, Ingram LO (2001). Gene integration and expression and extracellular secretion of *Erwinia chrysanthemi* endoglucanase celY (celY) and celZ (celZ) in ethanologenic *Klebsiella oxytoca* P2. Appl. Environ. Microbiol. 67:6-14.