Ann Microbiol (2013) 63:1257–1263
DOI 10.1007/s13213-012-0583-8

ORIGINAL ARTICLE

Utilization of renewable agricultural residues for the production of extracellular halostable cellulase from newly isolated *Halomonas* sp. strain PS47

Pooja Shivanand · Gopal Mugeraya · Anubhav Kumar

Received: 18 September 2012 / Accepted: 29 November 2012 / Published online: 20 December 2012

Abstract A newly isolated biopolymer-degrading halophilic bacterium, *Halomonas* sp. strain PS47, yielded higher cellulase activity (0.0076 U/ml) in mineral salt medium (MM63). Activity increased to 0.029 U/ml when carboxymethyl cellulose (0.5 % w/v) was used as carbon source and further to 0.138 U/ml when a combination of yeast extract and peptone was used as nitrogen source. Enzyme secretion was maximal during late exponential and stationary phases (0.15 U/ml, 48 h). Among different agro-residues (1 % w/v), wheat bran gave the highest activity (0.12 U/ml) at pH 7.5, 30 °C and 6 % (w/v) NaCl. The cellulase exhibited higher activity at pH 7.1 and 50 °C. The enzyme exhibited activity over a wide range of NaCl concentrations (0–4 M). Optimum activity was at 0–1 M NaCl. At 4 M NaCl, activity was reduced to 65 % of the initial value. The present investigation thus contributes to the limited information available on halostable cellulases.

Keywords *Halomonas* sp. · Moderately halophilic bacteria · Halostable cellulase · Agricultural residues · Alkaline pretreatment · Wheat bran

Introduction

Cellulose, a homopolymer of anhydro-D-glucose linked by β-1,4 bonds, is known to be the most abundant organic compound in the world (Huang and Monk 2004). Recently, the potential of cellulases [endo-1,4-b-glucanase (EC3.2.1.4)], a group of hydrolytic enzymes which hydrolyze the glucosidic bonds of cellulose (Kim et al. 2005), has been revealed in various industries, such as food, textiles and laundry, pulp and paper, and agricultural (Kang et al. 2004; Long et al. 2009). However, industrial applications have mainly focused on using fungal enzymes with less emphasis on bacterial cellulases (Ulrich et al. 2008). The industrial applications of cellulases are hampered by high production costs owing to the use of pure chemicals coupled with low enzyme activities. Renewable lignocellulosic biomass is now considered to be potential alternate energy source (Aristidou and Penttila 2000).

Enzymes active over a high range of salinity can be obtained from halophilic species. Halophilic bacteria form a versatile group adapted to life at varying concentrations of salt, unlike the obligate halophilic archaea which compulsorily require high salt concentrations for growth and survival (Ventosa et al. 1998). Halophilic microbial species have the potential to yield valuable new products for biotechnology (Margesin and Schinner 2001; Shivanand and Mugeraya 2011). Halophilic enzymes are often also thermotolerant and alkalitolerant and hence polyextremophilic (Moreno et al. 2009). Halophilic or halostable cellulase can be used in high-salt and high osmotic pressure environments thereby reducing water consumption. They have great potential in treatment of agricultural wastes and bioremediation of cellulose materials in conditions of low water activity. In spite of the potential benefits, there is very limited information about cellulases from halophilic microorganisms. Only recently, a salt-activated cellulase from a halotolerant, alkaliphilic bacterium *Bacillus agaradhaerens* (Hirasawa et al. 2006), halophilic and alkaliphilic *Bacillus* sp. BG-CS10 (Zhang et al. 2012), and a cellulase with endoglucanase-like activity from a halophilic bacterium *Salinivibrio* sp. strain NTU-05 (Wang et al. 2009) have been reported.

P. Shivanand (✉) · G. Mugeraya · A. Kumar
Department of Chemical Engineering, National Institute of Technology Karnataka, Surathkal 575025, India
e-mail: poojashivanand@nitk.ac.in

P. Shivanand
e-mail: pooja.shivanand@gmail.com

In salt marsh ecosystems, abundant lignocellulosic polymers are degraded by microorganisms as an essential component of food web and carbon cycling processes (Kristensen et al. 2008). Most saline environments have an influx and presence of plant matter and, hence, the resident microbes can be expected to harbor the machinery for the biomass exploitation. Agricultural residues (lignocellulosic biomass) are mainly composed of (on dry weight basis)—cellulose (40–60 %), hemicellulose (xylan, 20–40 %) and lignin (10–25 %) (Saha 2003). For cellulase production, lignocelluloses are generally pretreated to release cellulose. The purpose is to remove lignin and increase the porosity of the materials (Dobrev et al. 2007). Submerged cultivation using agricultural residues is used more widely for bacterial cultures, allowing a better level of process intensification and automation.

Utilization of agricultural residues for the production of saline hydrolases from halophilic bacteria has been less attempted. Use of cheap agricultural residues can substantially reduce production costs and in turn even provide value to the waste.

Materials and methods

Chemicals and experimental statistics

All chemicals used were of analytical grade and media components of highest purity grade. The microbiological media used were dehydrated media. Production studies were carried out as batch cultures in 250-ml Erlenmeyer flasks, containing 100 ml of culture media. Agricultural residues were procured from processing mills and local market. All the experiments were carried out independently in triplicate and repeated twice. The standard deviation in results was within experimental limits.

Bacterial isolation and screening for cellulase activity

The salt-enriched soil and water samples were collected from salterns, coastal shores, and salt marsh ecosystems of west coast of Karnataka, India. Bacterial isolation was done by the serial dilution plating technique on MM63 (pH 7.1) containing in g/l: KH_2PO_4, 13.61; KOH, 4.21; $(NH_4)_2SO_4$, 1.98; $MgSO_4 \cdot 7H_2O$, 0.25; $FeSO_4 \cdot 7H_2O$, 0.0011; and NaCl, 60 in 1,000 ml water. The carbon source glucose in the mineral medium was replaced by CMC (5 g/l).

For screening cellulase producers, the bacterial isolates were grown in MM63 broth at pH 7.1 and 30 °C for 48 h. Supernatants from these cultures were incubated in the wells of CMC (1 %, w/v) enriched agar plates. After incubation of the plates for 24 h, zones of hydrolysis were visualized by staining the plates with aqueous solution of 0.1 % (w/v) Congo red for 15 min, and then destained with 1 M NaCl (Teather and Wood 1982). Morphological and physiological characteristics of the potential strain PS47 were studied. The strain was further identified by 16S rRNA gene amplification and nucleotide sequencing.

Inoculum preparation and enzyme assay

A 1 % inoculum from an overnight grown culture in log phase was added to 100 ml MM63 medium taken in 250-ml Erlenmeyer flasks. After incubation for 48 h, at 30 °C, under shaking condition of 150 rpm, the culture was harvested and growth was measured as OD_{600}, spectrophotometrically. The cultures were centrifuged at 10,000 rpm for 10 min at 4 °C. The cell-free extract was used as crude preparation to measure cellulase activity.

For enzyme assay, CMC was used as the substrate. Enzyme activity was determined by measuring the release of reducing sugars during the enzyme substrate reaction using dinitrosalicylic acid method (Ghose 1987). The values were determined from glucose standard curve. One unit (IU) of activity was defined as the amount of enzyme required to liberate 1 μmol of glucose per minute under given assay conditions.

Cellulase production on MM63 medium

Cellulase production was investigated in MM63 medium by replacing the carbon source glucose with CMC and nitrogen source $(NH_4)_2SO_4$ with organic sources like yeast extract and peptone. Different agro-residues like wheat bran, rice bran, corn cobs, sugarcane bagasse, groundnut shells, and sunflower seeds were checked for the source of carbon in the medium at 1 % (w/v) for cellulase production. The culture was grown at pH 7.1 and temperature 30 °C at 150 rpm for 48 h before estimation of enzyme activity. Cellulase activity was checked in both treated and untreated agro-residue media. The activity was also checked in media supplemented with yeast extract and peptone as nitrogen sources.

Alkaline treatment of agricultural residues

Alkaline treatment of agro-residues was carried out according to the method described by Pham et al. (1998). The residues were ground to particles of 0.5–1 cm. For de-lignification, 100 g of these agro-residues taken in 1-l Erlenmeyer flasks were soaked in 1 % NaOH and autoclaved at 121 °C for 20 min. After the alkali treatment, the materials were washed with tap water until neutral and oven-dried. Treated substrates were passed through 0.5-mm screens for use as medium component.

Growth and cellulase production in media with different salt concentration

The effect of salt on growth and cellulase secretion was studied by varying the NaCl concentration from 0 to 15 %

(w/v) in the wheat bran medium. The initial pH of the medium was adjusted to 7.1. Growth and cellulase activity of PS47 were monitored every 4 h, at 30 °C and 150 rpm for a period of 72 h.

Effect of pH and temperature of the medium for cellulase production

In order to investigate the influence of pH on growth and cellulase production, the isolate, PS47 was grown in MM63-wheat bran medium containing 6 % (w/v) NaCl at different pH (3.0–13.0) and constant temperature of 30 °C. After 48 h, cellulase activity was quantified. Similarly, influence of temperature was investigated by varying the growth temperature (25–50 °C) at optimum pH, keeping the other parameters constant.

Properties of the extracellular cellulase

The optimum pH for cellulase activity was determined with CMC as substrate dissolved in the following buffer systems: potassium phosphate buffer (pH 6.0–8.0) and glycine-NaOH buffer (pH 9.0–12.0). The optimum temperature was determined for the cellulase at different temperatures (10–70 °C). For the study of halostability, the enzyme was pre-incubated with NaCl (0–4 M) at 30 °C for 1 h and the enzyme activity was determined.

Results and discussion

Selection of the cellulase-producing bacterium

Moderately halophilic bacteria were isolated from the coastal regions and salt marsh ecosystems of west coast of Karnataka, India, on MM63 medium containing 6 % (w/v) NaCl and CMC as the sole source of carbon. Bacteria growing on this biopolymer medium were expected to harbor the potential enzyme machinery to degrade CMC and utilize the polymer for growth and multiplication. Based on colony characteristics, 16 different organisms (PS33-PS48, numbered serially) were isolated, cultured aerobically in the same medium (pH 7.1) at 30 °C, and maintained as pure cultures in agar stabs and glycerol stock. From these, 6 strains showed prominent zone of clearance on the CMC agar indicating extracellular cellulolytic activity. The following enzyme activities were recorded (per ml): PS33 (0.075 U), PS36 (0.089 U), PS37 (0.087), PS41 (0.0049), PS43 (0.003 U), PS44 (0.07 U), and PS47 (0.14 U). Therefore, based on the highest enzyme activity, PS47 was selected for further investigation. The strain was isolated from the Baad–Gudeangadi salt marsh ecosystem in Kumta region, Karnataka, India.

Characteristics of the potential strain

Strain PS47 is a Gram-negative, short rod, which formed circular, convex, pale yellow-colored, mucoid colonies on the agar surface. Cells were encapsulated and motile, occurring singly or in pairs. Growth occurred with 0.5–15 % (w/v) NaCl (optimally with 6 %, w/v), at pH 7.0–10.0 (optimally at pH 7.1) with an optimal growth temperature of 30 °C.

According to *The Bergey's Manual of Determinative Bacteriology* and on the basis of 16S rRNA gene studies, strain PS47 has been identified as *Halomonas* sp. (Fig. 1). The ribosomal RNA gene sequence has been submitted to GenBank (ID: JQ425853). *Halomonas* sp. strain PS47 is a relatively novel marine bacterium.

The study of growth kinetics of *Halomonas* sp. PS47 with reference to cellulase production in MM63 medium indicated that the lag phase of the organism was small up to 3 h, after which the growth was exponential up to 24 h followed by stationary phase. Cellulase secretion was maximal in the late exponential and early stationary phase (30–54 h) with the highest activity recorded at 48 h (0.0076 U/ml) in the basal medium. Zone of clearance in the screen-plate and optimum levels of cellulase secretion in the basal MM63 medium indicate cellulolytic activity as an inherent property of *Halomonas* sp. PS47.

Cellulase production in MM63 medium

Cellulase production was investigated in the basal MM63 medium in the presence of CMC, yeast extract, and peptone

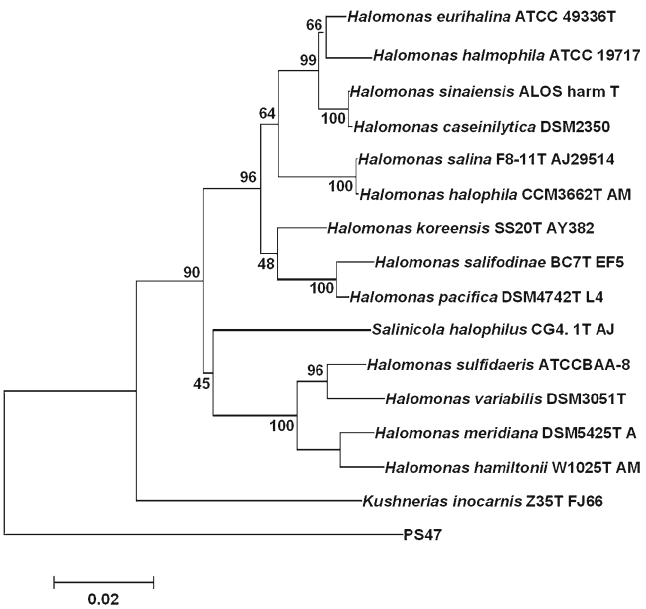

Fig. 1 Neighbor-joining tree showing the position of isolate *Halomonas* sp. PS47 to a selected number of members of the halophilic bacteria. Values are the branch lengths reflecting the actual distances between the sequences

(Fig. 2a). The enzyme activity increased around 5 times to 0.036 U/ml when the medium containing glucose was supplemented with complex nitrogen sources like yeast extract and peptone in place of the inorganic nitrogen source $(NH_4)_2SO_4$. When CMC was used as carbon source instead of glucose and $(NH_4)_2SO_4$ as the nitrogen source in the basal medium the enzyme activity was found to be 0.03 U/ml, 4 times the original value. However, only yeast extract and peptone in the absence of any other carbon source failed to induce substantial enzyme production (0.0056 U/ml). The highest activity (0.14 U/ml) was seen when glucose in MM63 medium was replaced by CMC and $(NH_4)_2SO_4$ was replaced by a combination of yeast extract and peptone as the organic nitrogen sources. The activity was 18-fold higher than the original value. The enzyme secretion corresponded with growth and was higher in late exponential and stationary phases of growth (Fig. 2b). This shows that CMC acts as an inducer while yeast extract and peptone enhance the cellulase enzyme production by *Halomonas* sp. PS47. Cellulase production is controlled by catabolite repression and induction, as

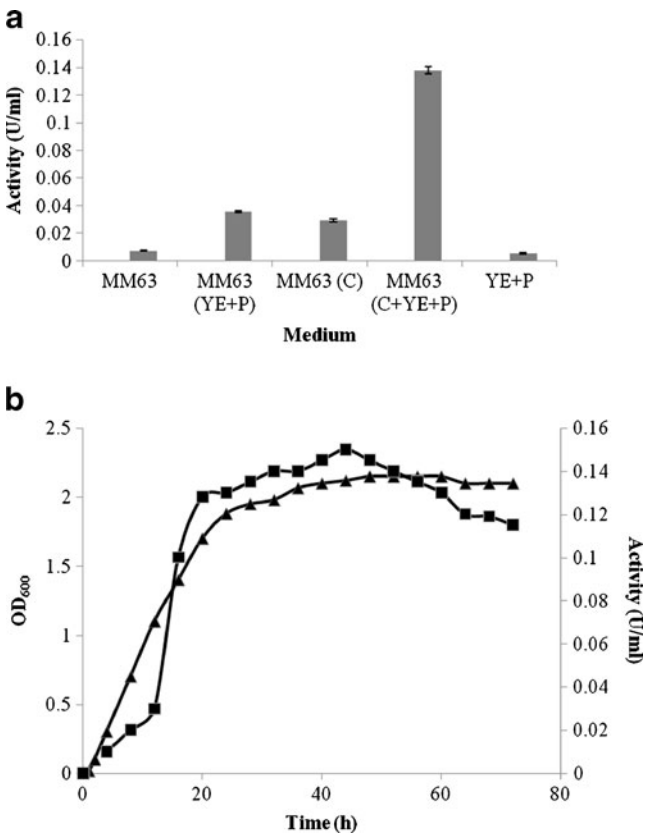

Fig. 2 **a** Cellulase production of *Halomonas* sp. PS47 in MM63 medium. *C* CMC, *P* peptone, *YE* yeast extract. Enzyme activity was determined after 48 h of bacterial growth in MM63 containing 6 % (w/v) NaCl. **b** Growth curve and cellulase secretion of *Halomonas* sp. PS47 in MM63 medium containing CMC. Samples were withdrawn at 4-h intervals for the determination of cell growth by OD_{600} (*black triangle*) and cellulase activity (*black square*)

reported for the alkaline cellulases from *Bacillus* sp. KSM-19, KAM-64, and KSM-520 (Shikata et al. 1990). The production of cellulases is induced in the presence of substrate and is repressed in the presence of easily utilizable sugars in the medium.

The cellulolytic capabilities of different microorganisms are highly variable. Although the reported activity is lower compared to the activities of some fungal strains, it is substantial when compared to those reported from bacterial species. Every microorganism has its own special condition for growth and metabolite production. Optimization of the medium components and growth conditions using statistical methodologies would result in enhanced production of cellulase. Deka et al. (2011) reported cellulase activity of *Bacillus subtilis* AS3 in the unoptimised CMC medium to be 0.07 U/ml. By optimizing the medium further (CMC, peptone and yeast extract), a six-fold increase in enzyme activity was obtained (0.43 U/ml). A maximum cellulase activity of 0.043 U/ml has been reported from cell-free culture supernatants of *Geobacillus* sp. isolated from a deep goldmine environment (Rastogi et al. 2009). Li et al. (2008) reported maximum cellulase activity (0.26 U/ml) of a *Bacillus* sp. when the culture was grown in LB medium supplemented with 1 % CMC. In another study, a cellulase activity of 0.0113 U/ml was observed under optimized conditions from *Geobacillus* sp. (Tai et al. 2004). These studies give a brief idea of the different cellulolytic potentials of microorganisms. In general, higher production of enzyme requires the presence of complex nitrogen sources. Yeast extract and peptone have been reported to have a significant effect on cellulase production. Yeast extract is known to be an effective medium component for the growth of halophilic bacteria (Shivanand and Jayaraman 2009). A low-cost fermentation medium can be designed for the production of halostable cellulase by using agricultural by-products. These complex carbohydrate sources can serve as basal and optimized medium for obtaining higher yields of the enzyme.

Cellulase production on different agricultural residues

Among the different agricultural residues used, wheat bran was found to yield the highest amount of cellulase (Fig. 3a). Rice bran and cobs also yielded substantial amounts of cellulase. Lower amounts of cellulase activity were detected when sugarcane bagasse and groundnut shells were used as the carbon source. Very low enzyme units were recorded when sunflower seeds were used in the medium.

Alkaline pretreatment of agro-residues proved to be very effective in providing a good growth medium for enhanced production of cellulase from PS47. Pretreatment solubilizes hemiculloses and decreases crystallinity of the substrate. It has been reported that compared to acid or oxidative

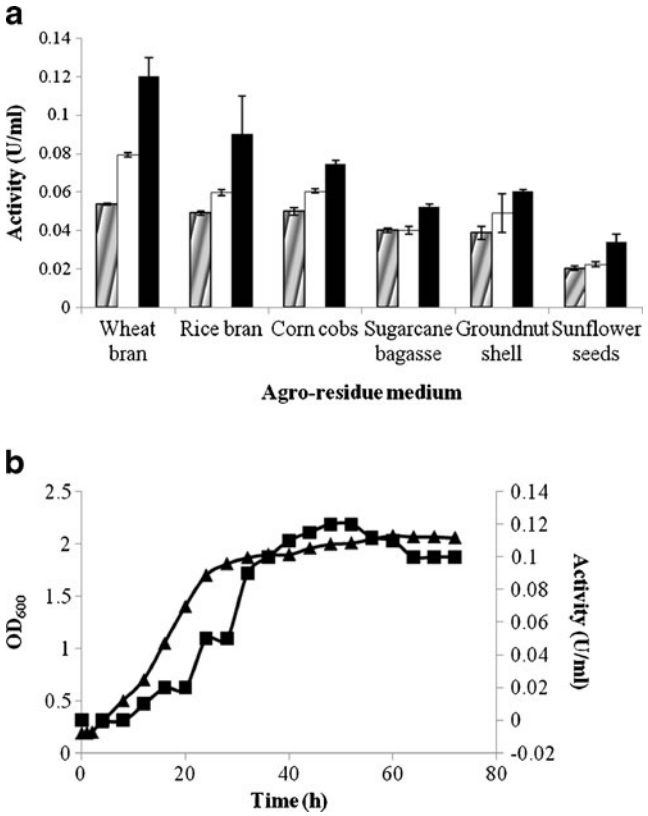

Fig. 3 **a** Cellulase production of *Halomonas* sp. PS47 in MM63 medium containing untreated agro-residue (*hatched column*); treated agro-residue (*white*) and treated agro-residue (*black*) along with yeast extract and peptone. **b** Growth curve and cellulase secretion of *Halomonas* sp. PS47 in MM63 medium containing wheat bran. Samples were withdrawn at 4-h intervals for the determination of cell growth by OD_{600} (*black triangle*) and cellulase activity (*black square*)

reagents, alkali treatment appears to be the most effective method in breaking ester bonds between lignin, hemicellulose and cellulose, and avoiding fragmentation of hemicellulose polymers (Gaspar et al. 2007). In large-scale operations, the alkalis can be removed with large amounts of water, or neutralized with acidic solutions, which produces large amounts of salt. A possible advantage of using halophilic bacteria is that the salt created after neutralization does not need to be removed. Halo-alkaliphilic bacteria may provide an opportunity to avoid this costly step of neutralization which is cost effective on an industrial scale.

The addition of yeast extract and peptone to the pretreated wheat bran medium resulted in over two-fold increase in activity to 0.12 U/ml. The enzyme secretion in the wheat bran medium was higher in the stationary phase of growth (Fig. 3b). Higher production of enzyme requires the presence of complex nitrogen sources (Sing et al. 2001). Utilization of cellulosic wastes as cheap carbon and energy sources can serve as alternative to our rapidly depleting finite sources of fossil fuels. Halophilic bacteria capable of utilizing cheap renewable agricultural wastes could be exploited as an economic alternative to existing production processes. Utilization of pure carbon sources is impractical for commercial production of enzymes owing to their high costs. The production of cellulase demonstrated in this study can be used for the hydrolysis of lignocellulosic biomass which can in turn be used for the cost-effective production of ethanol.

Growth and cellulase production in media with different salt concentration

Growth (Fig. 4a) in media with different concentrations of NaCl followed a similar pattern, where exponential phase was observed from 4 to 24 h followed by stationary phase. The bacterium was able to grow well up to a NaCl concentration of 15 % (w/v). Efficient growth was not seen at higher concentrations of NaCl. Growth was optimal at NaCl concentrations in the range of 3 %–9 % (w/v) where the bacterium followed similar growth patterns. Cellulase

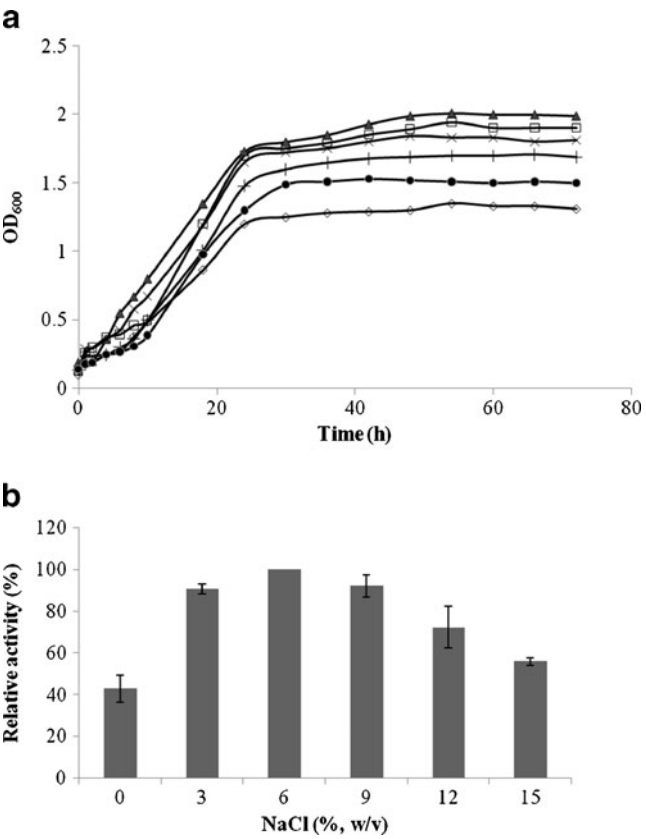

Fig. 4 **a** Growth curves of *Halomonas* sp. PS47 in media with different salt media, % (w/v): 0 (*white diamond*); 3 M (*white square*); 6 M (*black triangle*); 9 (*cross*); 12 (*plus sign*) and 15 (*black circle*). Samples were withdrawn at every 6-h interval for the determination of cell growth (OD_{600}). **b** Cellulase production of *Halomonas* sp. PS47 in different salt media. The highest activity in 6 % (w/v) NaCl medium is taken as 100 %

production (Fig. 4b) was also higher in this range of NaCl (3–9 %, w/v), the optimum being at 6 % (w/v). Though growth was much stunted at 12 and 15 % (w/v) NaCl, the bacterium was able to produce extracellular cellulase. These studies confirm the moderately halophilic nature of the bacterium and its versatility in adaptations to increasing levels of salinity.

Effect of pH and temperature

Halomonas sp. PS47 could grow and produce extracellular cellulase over a wide range of pH (6.0–10.0). Higher activity was seen in the pH range of 6.5–8. Maximum cellulase production was obtained at pH 7.5 (Fig. 5a). The optimum temperature for cellulase production was 30 °C (Fig. 5b). Production was significantly reduced at 45 °C, which was not favorable for growth.

Properties of the extracellular cellulase

The cellulase was active in the pH range of 7.0–10.0, with an optimum at pH 7.1 (Table 1). The enzyme had maximum cellulolytic activity at 50 °C, but is active over a wide range of temperature (35–65 °C). The enzyme exhibited appreciable activity in the presence of NaCl up to a concentration of 4 M, although the highest activity was seen in the presence of 0–1 M NaCl. The activity was reduced to 65 % of the original value at 3–4 M NaCl. Halostable enzymes constitute an excellent model for structural adaptations. Although halophilic enzymes display identical enzymatic functions as their non-halophilic counterparts, they have different properties, including stability and activity at different ranges of salinity (Amoozegar et al. 2007). Halostability is an important characteristic that will facilitate the future application of the cellulase in biotechnological processes containing high salinity or osmotic pressures. The ability of the moderately halophilic bacteria to survive and produce enzymes that are active over a very wide range of salinities made them attractive candidates for the isolation of novel enzymes.

Table 1 Properties of the extracellular cellulase produced by *Halomonas* sp. strain PS47

pH optimum	7.1
T optimum	50 °C
NaCl stability	
0 M	0.12±0.00057 U/ml
0.5 M	0.117±0.0006 U/ml
1 M	0.115±0.003 U/ml
1.5 M	0.099±0.0011 U/ml
2 M	0.087±0.0025 U/ml
3 M	0.079±0.001 U/ml
4 M	0.0783±0.003 U/ml

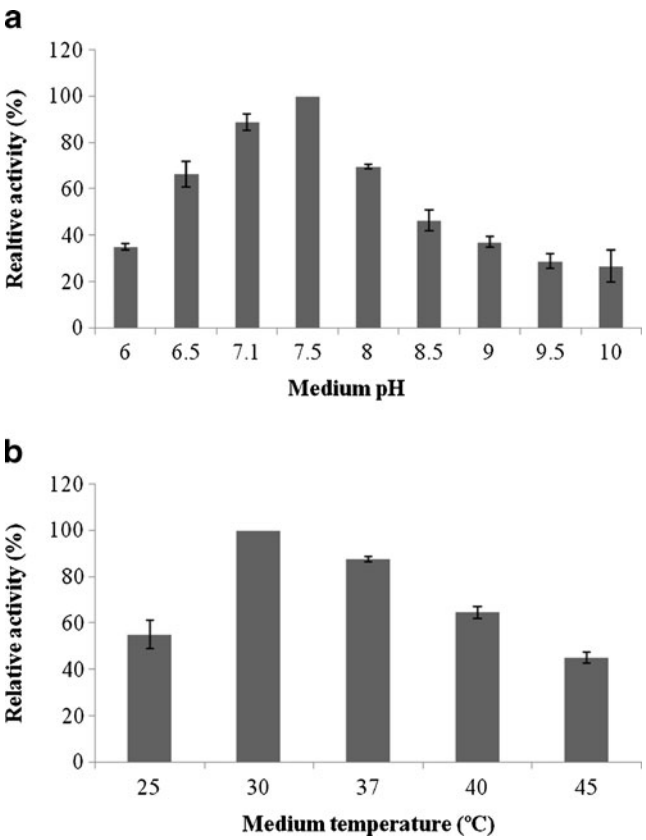

Fig. 5 **a** Effect of medium pH on cellulase production by *Halomonas* sp. PS47. Samples were taken after incubation of 48 h at 30 °C, for the determination of cellulase activity (U/ml). Highest production at pH 7.5 is taken as 100 %. **b** Effect of medium temperature on cellulase production by *Halomonas* sp. PS47. Samples were withdrawn after incubation of 48 h at pH 7.5, for the determination of cellulase activity (U/ml). Highest production at 30 °C is taken as 100 %

Conclusion

The present investigation assumes significance in the production of extracellular halostable cellulase from a newly isolated halophilic bacterium, *Halomonas* sp. strain PS47, using renewable agricultural residues like wheat bran. The bacterium is able to utilize different agro-residues for cellulase production. Moreover, growth and production are favorable over a wide range of NaCl concentrations. The thermo-stable, salt and pH-tolerant cellulase is a promising candidate for industrial applications. Few reports are available on the usage of renewable agricultural resources for the growth of halophiles. Thus, halophilic bacteria capable of utilizing cheap agricultural wastes could be exploited as an economic alternative to existing production processes.

Acknowledgment Financial Assistance from National Institute of Technology Karnataka, Surathkal (Mangalore, India) is gratefully acknowledged.

References

Amoozegar MA, Salehghamari E, Khajeh K, Kabiri M, Naddaf S (2007) Production of an extracellular alkaline metalloprotease from a newly isolated, moderately halophile, *Salinivibrio* sp. strain AF-2004. Microbiol Res 162:369–377

Aristidou A, Penttila M (2000) Metabolic engineering applications to renewable resource utilization. Curr Opin Biotechnol 11:187–198

Deka D, Bhargavi P, Sharma A, Goyal D, Javed M, Goyal A (2011) Enhancement of cellulase activity from a new strain of *Bacillus subtilis* by medium optimization and analysis with various cellulosic substrates. Enzym Res, Article ID 151656, 8 pages. doi:10.4061/2011/151656

Dobrev GT, Pishtiyski IG, Stanchev VS, Mircheva R (2007) Optimization of nutrient medium containing agricultural wastes for xylanase production by *Aspergillus niger* B03 using optimal composite experimental design. Bioresour Technol 98(14):2671–2678

Gaspar M, Kalman G, Reczey K (2007) Corn fiber as a raw material for hemicellulose and ethanol production. Process Biochem 42:1135–1139

Ghose TK (1987) Measurement of cellulase activities. Pure Appl Chem 59:257–268

Hirasawa K, Uchimura K, Kashiwa M, Grant WD, Ito S, Kobayashi T, Horikoshi K (2006) Salt-activated endoglucanase of a strain of alkaliphilic *Bacillus agaradhaerens*. Antonie Van Leeuwenhoek 89:211–219

Huang XP, Monk C (2004) Purification and characterization of a cellulase (CMCase) from a newly isolated thermophilic aerobic bacterium *Caldibacillus cellulovorans* gen. nov., sp. nov. World J Microbiol Biotechnol 20:85–92

Kang SW, Park YS, Lee JS, Hong SI, Kim SW (2004) Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour Technol 91:153–156

Kim JY, Hur SH, Hong JH (2005) Purification and characterization of an alkaline cellulase from a newly isolated alkalophilic *Bacillus* sp. HSH-810. Biotechnol Lett 27:313–316

Kristensen E, Bouillon S, Dittmar T, Marchand C (2008) Organic carbon dynamics in mangrove ecosystems: a review. Aquat Bot 89:201–219

Li W, Zhang WW, Yang MM, Chen YL (2008) Cloning of the thermostable cellulase gene from newly isolated *Bacillus subtilis* and its expression in *Escherichia coli*. Mol Biotechnol 40:195–201

Long C, Ou Y, Guo P, Liu Y, Cui J, Long M, Hu Z (2009) Cellulase production by solid state fermentation using bagasse with *Penicillium decumbens* L-06. Ann Microbiol 59(3):517–523

Margesin R, Schinner F (2001) Potential of halotolerant microorganisms for biotechnology. Extremophiles 5:73–83

Moreno MDL, Garcia MT, Ventosa A, Mellado E (2009) Characterization of *Salicola* sp. IC10, a lipase- and protease-producing extreme halophile. FEMS Microbiol Ecol 68:59–71

Pham PL, Taillandier P, Delmas M, Strehaiano P (1998) Production of xylanases by *Bacillus polymyxa* using lignocellulosic wastes. Ind Crop Prod 7:195–203

Rastogi G, Muppidi GL, Gurram RN, Adhikari A, Bischoff KM, Hughes SR, Apel WA, Bang SS, Dixon DJ, Sani RK (2009) Isolation and characterization of cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine, Lead, South Dakota, USA. J Ind Microbiol Biotechnol 36:585–598

Saha BC (2003) Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30:279–291

Shikata S, Saeki K, Okoshi H, Yoshimatsu T, Ozaki K, Kawai S, Ito S (1990) Alkaline cellulase laundry detergents: production by alkalophilic strains of *Bacillus* and some properties of the crude enzymes. Agric Biol Chem 54:91–96

Shivanand P, Jayaraman G (2009) Production of extracellular protease from halotolerant bacterium, *Bacillus aquimaris* strain VITP4 isolated from Kumta coast. Process Biochem 44:1088–1094

Shivanand P, Mugeraya G (2011) Halophilic bacteria and their compatible solutes—osmoregulation and potential applications. Curr Sci 100:1516–1521

Sing J, Batra N, Sobti RC (2001) A highly thermostable, alkaline CMCase produced by a newly isolated *Bacillus* sp. VG1. World J Microbiol Biotechnol 17:761–765

Tai SK, Lin HPP, Kuo J, Liu JK (2004) Isolation and characterization of a cellulolytic *Geobacillus thermoleovorans* T4 strain from sugar refinery wastewater. Extremophiles 8:345–349

Teather RM, Wood PJ (1982) Use of Congo-red polysaccharide interaction in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43:777–780

Ulrich A, Klimke G, Wirth S (2008) Diversity and activity of cellulose-decomposing bacteria, isolated from a sandy and a loamy soil after long-term manure application. Microb Ecol 55:512–522

Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544

Wang CY, Hsieh YR, Ng CC, Chan H, Lin HT, Tzeng WS, Shyu YT (2009) Purification and characterization of a novel halostable cellulase from *Salinivibrio* sp. strain NTU-05. Enzyme Microb Technol 44:373–379

Zhang G, Li S, Xue Y, Mao L, Ma Y (2012) Effects of salts on activity of halophilic cellulase with glucomannanase activity isolated from alkaliphilic and halophilic *Bacillus* sp. BG-CS10. Extremophiles 16:35–43