ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 6, pp. 736–742. © Pleiades Publishing, Ltd., 2012.

EXPERIMENTAL ARTICLES

Screening and Identification of Newly Isolated Cellulose-Degrading Bacteria from the Gut of Xylophagous Termite *Microcerotermes diversus* (Silvestri)[1]

Z. Pourramezan[a, 2], G. R. Ghezelbash[a, b], B. Romani[c], S. Ziaei[d], and A. Hedayatkhah[a]

[a] *Department of Biology, Faculty of Science, Shahid Chamran University, Ahwaz , 61357-43135 Iran*

[b] *Department of Biology, Faculty of Science, Isfahan University, Isfahan, Iran*

[c] *Division of Medical Virology, Department of Pathology, University of Stellenbosch, Tygerberg, 7505 South Africa*

[d] *Department of Biotechnology, Faculty of Advanced Science and Technology, Isfahan University, Isfahan, Iran*

Received November 18, 2011

Abstract—The aim of the present study was to isolate and characterize the cellulose-degrading bacteria from the gut of the local termite, *Microcerotermes diversus* (Silvestri), inhabiting the Khuzestan province of Iran. The microorganisms capable of growing in the liquid medium containing cellulose as the only source of carbon were isolated and their cellulolytic activity on CMC-containing media was confirmed by the congo red clearing zone assay. The isolates were identified based on biochemical characteristics and the phylogenetic analysis of 16S rRNA gene fragments. The results of the present study show that three cellulose-degrading bacteria isolated from local termite guts belonged to the genera *Acinetobacter, Pseudomonas* and *Staphylococcus* and four cellulose-degrading bacteria belonged to *Enterobacteriaceae* and *Bacillaceae* families. Several isolates recovered from separate termite *Microcerotermes diversus* samples closely clustered in phylogenetic trees indicating high similarity and the abundance of particular cellulolytic strains. *Bacillus* B5B and *Acinetobacter* L9B hydrolyzed cellulose faster than the other isolates (with CMCase activity of 1.47 and 1.22 U/mL, respectively). The stability of CMCase produced by *Bacillus* B5B over a broad range of pH and high temperature indicated that the enzyme may be of great commercial value.

Keywords: 16S rRNA gene, cellulose-degrading bacteria, *Microcerotermes diversus*, phylogenetic analysis, termite

DOI: 10.1134/S0026261712060124

Cellulases have attracted much interest because of the diversity of their applications [1, 2]. However, the major bottleneck of comprehensive application of cellulase in industry is the high cost of the enzyme production. Substantial cost reduction may be possible by exploring ways of cellulose conversion using microorganisms that produce cellulolytic enzymes. It is therefore imperative to look for microorganisms that have a high rate of cellulase production [3].

Cellulase research has been concentrated mostly in fungi but there is increasing interest in cellulase production by bacteria [4]. Bacteria that have a high growth rate as compared to fungi have a good potential to be used in cellulase production. Bacterial cellulases are more effective catalysts. However, bacteria are not widely used for cellulase production. Isolation of a novel microbial strain, having hyper productivity of cellulase with more activity and high stability against temperature and pH might make the process economically attractive [1].

Bacteria inhabit a wide variety of environmental and industrial niches, which produce cellulolytic strains that are extremely resistant to environmental stresses [5].

It has been suggested that termite gut microbiota has a significant impact on cellulose degradation [6]. In fact, in tropical and subtropical regions termites play an important role in the degradation of organic matter and in the turnover of complex biopolymers, such as wood and other cellulose- and hemicellulose-containing materials [7].

The gut microbiota enables termites to efficiently hydrolyze cellulose. The cellulase activity of termite hindgut is attributed to cellulose-degrading bacteria [8]. Termites are diverse in their feeding habits that lead to diverse microbiotas.

In this study, the diversity of culturable aerobic and facultatively anaerobic cellulose-degrading bacteria in the gut of local termite *Microcerotermes diversus* (Silvestri) is addressed that has not been described yet, in order to obtain a more precise idea of their occurrence in termites and determine their ability to yield high

[1] The article is published in the original.

[2] Corresponding author; e-mail: z.pourramezan@gmail.com

levels of extracellular cellulases to use as probable application in the industry.

MATERIAL AND METHODS

Termites. Twenty adult termite workers *Microcerotermes diversus* (Silvestri) were collected from decomposing logs. Separate termite colonies near the Shahid Chamran University of Ahwaz, Iran, were used for collecting termite samples. These termites are widely distributed throughout the khuzestan province.

Screening of cellulose-degrading bacteria. For the isolation of the gut bacteria, termites were surface sterilized with 70% ethanol and washed with sterile distilled water. All further steps were performed under sterile conditions. Ten entire guts were removed from the freshly killed termites and disrupted by passing through a syringe needle. The disrupted gut debris was suspended in 1 mL of mineral medium 1 containing 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.7% K_2HPO_4, 0.05% Na citrate. Medium 1 was supplemented either with filter paper strips (medium 1a) or 0.1% carboxymethylcellulose (medium 1b). The inoculated media were incubated aerobically and anaerobically at 30°C for up to 1 week.

Liquefaction of CMC or solubilization of filter paper was used as a primary indication of cellulases production. Microorganisms capable of growing in cellulose-containing media were isolated and their cellulolytic activity was confirmed by a congo red clearing zone assay. The zone of cellulose hydrolysis was apparent as a clear area in the otherwise congo red stain background in CMC agar plates [9, 10]. The ratio of the clear zone diameter to colony diameter was measured in order to select the highest cellulase activity producer [11].

The microorganisms with cellulolytic activity were subcultured further to get the pure colonies. Culture broth of cellulose-producer bacteria was sampled at different time during growth to determine cell density by measurement of absorbance at 600 nm and enzyme production by carboxymethyl cellulase hydrolysis.

Enzyme activity assay. Cellulose activity was measured by determination of reducing sugar released from CMC or filter paper through DNS (dinitrosalicylic acid) method [12]. For comparison between all isolates the same OD_{600} was used to eliminate the biomass effect on the evaluation.

Isolates were grown under optimal condition (pH 6.0, 37°C) for cellulase production. Cells were separated from the cultivation medium after centrifugation at 4800 rpm for 20 min at 4°C and the supernatant was used as the source of extracellular crude enzyme. Supernatants of all isolates were collected when the OD_{600} was 0.5, 0.7, 0.9, 1, 1.3 and 1.5. All these supernatants were incubated for 1 h at 40°C in 30 mL of 50 mM Na-acetate buffer (pH 5.5) with 2% CMC (Merck) for CMCase assay or whatman no. 1 filter paper strip (1 × 6 cm) for FPase assay. After 1 h the reaction was stopped by using of DNS (Merck). The amount of reducing sugar released in the hydrolysis was measured. Controls for carbohydrate produced from substrate and of the enzyme preparation were included. The supernatants at $OD_{600} = 1$ had the highest activity in most of the isolates, so this cell density was used for comparison between all of the isolates.

One unit of cellulase was defined as the amount of enzyme which produced 1 μmol glucose equivalent per hour under the assay conditions. The absorbance was measured with the WPA Biowave II UV/Vis Spectrophotometer (Biochrom, Cambridge, UK) at 546 nm. The amount of released reducing sugars was determined using a standard curve recorded for glucose.

The cellulases with higher activity were investigated for pH and temperature tolerance in order to use in industry. The influence of temperature on the catalytic activity of cellulases was determined by measuring the enzyme activity at temperature range from 30–80°C under the standard assay conditions. The influence of pH on the enzymatic activity was determined by measuring the enzyme activity at varying pH value ranging from 3 to 9 at optimum condition.

Identification of the bacterial isolates. Preliminary identification of isolates was performed using morphological and physiological characteristics. Physiological characteristics were determined based on several biochemical tests such as oxidase, catalase, motility, triple sugar iron (TSI), hydrogen sulphide (H_2S) and gas production, indole, methyl red, Simmon's citrate, and Voges Proskaeur. The results were compared with Bergey's Manual of Determinative Bacteria [13].

For molecular identification, the bacterial DNA was extracted using the Diatom DNA extraction kit (Genefanavaran, Iran) according to the protocol. The nearly full length 16S rRNA gene of the purified bacterial DNA was amplified using the eubacterial specific primers fD1 (5'-CCGAATTCGTCGACAACA GAG-TTTGATCCTGGCTCAG-3') and rP1 (5'-CC-CGGGATCCAAGCTTACGGTTACCTTGTTA-CGACTT-3') [14]. The amplification reactions contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, all four dNTPs (each at 0.2 mM), 50 pmol of each primer, 2.5 units of *Taq* DNA polymerase (Fermentas Inc., Glen Burnie, MD, United States), and 1–10 ng of template DNA in a 100-μL total reaction volume. A hot-start procedure (4 min, 94°C) was used before the enzyme was added to prevent nonspecific annealing of the primers. PCR was performed in a BioRad C1000™ thermal cycler (Bio-Rad Laboratories, Hercules, United States) with 30 cycles of 94°C for 1 min, 62°C for 30 s and 72°C for 1 min. This was followed by a final extension step of 7 min at 72°C and the PCR product was kept at 4°C. The PCR products were analyzed using agarose gel electrophoresis. PCR products were purified using the High Pure PCR

Table 1. 7 strains of environmentally isolated bacteria from the Gut of Termite *Microcerotermes diversus* with their accession numbers and cellulase activity (U/mL)

Isolate	Genus or family	GenBank accession number	CMCase (U/mL) at $OD_{600} = 1$	FPase (U/mL) at $OD_{600} = 1$
B2A	*Bacillaceae*	GU458349	0.73 ± 0.12	0.5 ± 0.16
L12	*Enterobacteriaceae*	GU458350	0.24 ± 0.11	0.17 ± 0.11
B3	*Staphylococcus*	GU458351	0.66 ± 0.11	0.49 ± 0.12
B5B	*Bacillaceae*	GU458352	1.47 ± 0.11	1.04 ± 0.13
L2	*Enterobacteriaceae*	GU458353	0.34 ± 0.12	0.27 ± 0.11
L8A	*Pseudomonas*	GU458354	0.84 ± 0.13	0.6 ± 0.15
L9B	*Acinetobacter*	GU458355	1.22 ± 0.11	0.86 ± 0.20

Table 2. Biochemical characterization of the isolates

Test	L2	L8A	L9B	L12	B5B	B3	B2A
Gram reaction	–	–	–	–	+	+	+
Shape	Rods	Rods	Rods	Rods	Rods	Cocci	Rods
Motility	–	–	–	–	+	+	+
Oxidase	–	+	–	–	–	–	–
Catalase	–	+	+	–	–	–	–
V-P reaction	–	–	–	–	–	–	–
Methyl red	+	–	–	+	+	+	–
Use of citrate	+	+	+	+	–	+	+
TSI	Acid/Alk	Alk/Alk	Acid/Alk	Acid/Alk	Acid/Alk	Alk/Alk	Acid/Alk
H_2S production in TSIA	–	–	–	–	–	–	–
Gas production in TSIA	–	–	–	–	–	–	–

Cleanup Micro Kit (Roche, Berlin, Germany) according to the manufacturer's suggestion. PCR products were directly sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit and analyzed on an ABI Prism 3130 automated DNA sequencer (Applied Biosystems, Foster City, CA).

The sequences were blasted using the NCBI genomic BLAST program by comparing each sequence with all available archaeal and bacterial sequences. Additional 16S rRNA gene sequences were obtained from the GenBank, EMBL, and DDBJ nucleotide sequence databases. All the sequences were aligned using Clustal X version 1.81 [15]. Multiple alignments were verified manually in BioEdit version 7.090. Neighbor-joining phylogenetic trees of gram-negative and gram-positive bacteria were separately constructed with MEGA version 4 [16]. The reliability of the branching and clustering pattern was estimated from 1000 bootstrap replicates.

The sequence data determined in this study were submitted to GenBank and published with the accession numbers GU458349 to GU458355.

RESULTS

Among the bacterial isolates, 7 strains were selected by the Congo red test. Upon further quantitative determination of cellulose degrading enzyme, all 7 isolates displayed activity of cellulase (CMCase) between 0.24–1.47 U/mL with the highest enzyme activity demonstrated by the isolate B5B. Table 1 shows all identified strains, their accession numbers and cellulase activity at $OD_{600} = 1$. Some biochemical parameters of these strains were investigated and the results were compared with Bergey's Manual of Determinative Bacteria (Table 2).

Four isolates identified as gram-negative bacteria were able to grow both in aerobic and anaerobic conditions and were considered facultative anaerobes.

L8A was a non-motile gram-negative bacillus with a big cream, mucoid colony on nutrient agar that clustered with *Pseudomonas putida* by more than 90% 16S rRNA gene sequence similarity, indicating that L8A belonged to the genus *Pseudomonas*. *Pseudomonas* L8A show a relatively high CMCase activity (0.84 ± 0.13 U/mL) at $OD_{600} = 1$.

L9B was a gram-negative, spherically shaped, aerobic and non-motile bacterium. Based on Bergey's

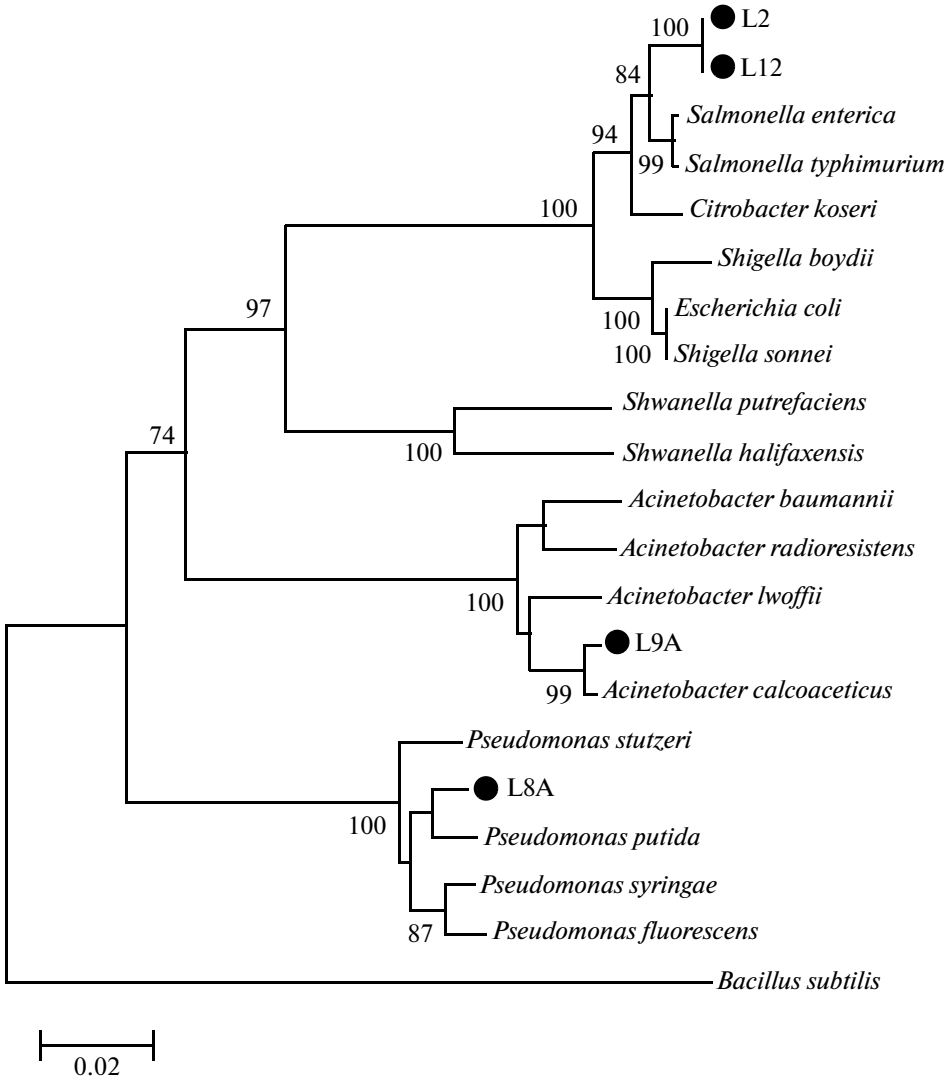

Fig. 1. Phylogenetic tree of the gram-negative isolates rooted with *B. subtilis* as the outgroup. The scale bar represents 0.02 substitutions per base position. Circles indicate the cellulose-degrading isolates. Bootstrap values above 70 from 1000 replicates are shown for each node.

manual and 16S rRNA sequence homology, L9B was identified as a member of *Acinetobacter* genus. *Acinetobacter* L9B showed the highest CMCase activity (1.22 ± 0.11 U/mL) among gram-negative bacteria.

L2 and L12 were characterized as gram-negative non-motile bacteria and identified as the members of *Enterobacteriaceae* family. The phylogenic analysis indicated that L2 and L12 cluster with the genus *Salmonella* by more than 80% 16S rRNA gene sequence similarity, confirming that these isolates belong to the *Enterobacteriaceae* family but not to any known species (Fig. 1). L2 and L12 isolates show the lowest CMCase activity among all isolates (0.34 ± 0.12 and 0.24 ± 0.11 U/mL, respectively).

Three isolates grew in aerobic conditions and all were identified as gram-positive bacteria.

B2A and B5B were both characterized as gram-positive, motile bacilli that clustered with *Bacillaceae* family by more than 80% 16S rRNA gene sequence similarity, but not with any known species (Fig. 2). B5B isolate showed the highest CMCase activity (1.47 ± 0.11 U/mL) among all the isolates. B3 was a gram-positive non-motile bacillus that closely clustered with *Staphylococcus warneri* by more than 80% 16S rRNA gene sequence similarity, suggesting that B3 belonged to the genus *Staphylococcus*. *Staphylococcus* B3 had relatively low CMCase activity (0.66 ± 0.11 U/mL).

Among all cellulases, those from *Bacillus* B5B were found to be thermostable up to 80°C. Presence of significant activity at 80°C (about 28% of maximum) and at 60°C (about 50% of maximum) indicated that this enzyme may be of great commercial value (Fig. 3). Industrial processes are generally carried out at elevated

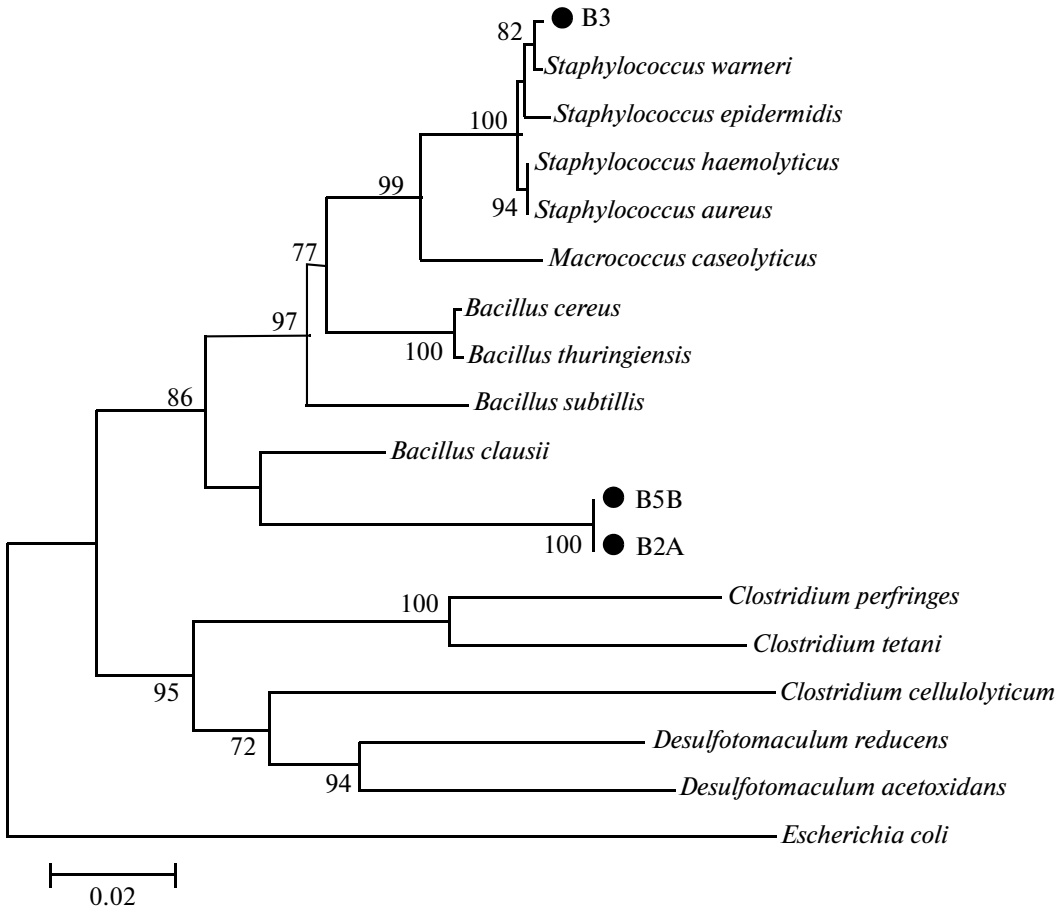

Fig. 2. Phylogenetic tree of the gram-positive isolates rooted with *E. coli* as the outgroup. The scale bar represents 0.02 substitutions per base position. Circles indicate the cellulose-degrading isolates. Bootstrap values above 70 from 1000 replicates are shown for each node.

temperature; therefore thermostable enzymes are required for industrial applications [17]. Thermostability profile of the enzyme showed that enzyme was thoroughly stable at 40 and 50°C. It was found that B5B isolate CMCase has activity over a broad range of pH (Fig. 4). Maximum activity was expressed at pH 6.

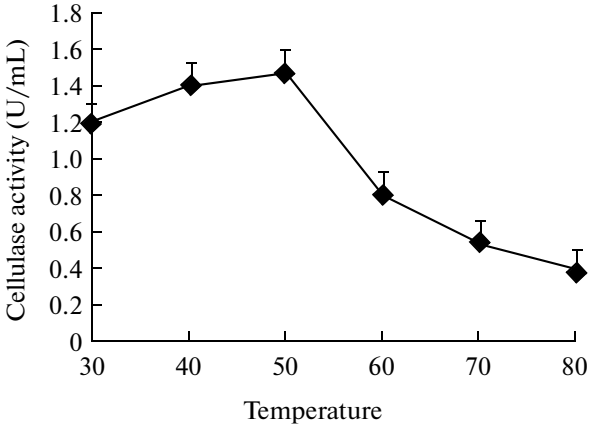

Fig. 3. Effect of temperature on the CMCase activity produced from *Bacillus* B5B.

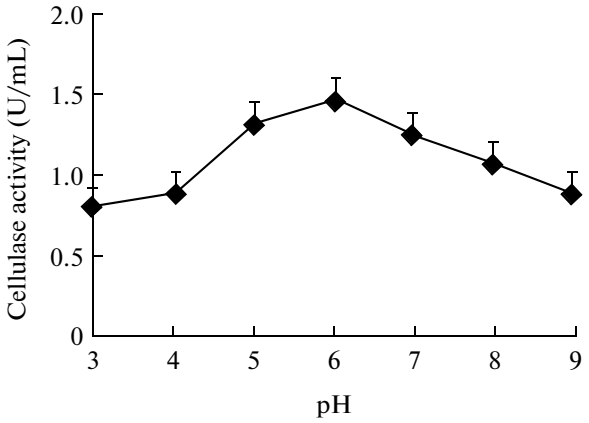

Fig. 4. Effect of pH-value on the CMCase activity produced from *Bacillus* B5B.

DISCUSSION

Although a large number of microorganisms can degrade cellulose, only a few of them produce significant quantities of free enzyme capable of completely hydrolyzing crystalline cellulose. Bacterial cellulases are either cell-bound or extracellular. In this work, a number of microorganisms capable to produce extracellular cellulases belonged to the gut microbiota of *Microcerotermes diversus* (Silvestri) was investigated.

Microcerotermes diversus Silvestri (*Isoptera: Termitidae*), an important pest in Ahwaz (Khouzestan, Iran) [18], is an extremely destructive structural wood pest, and is considered to be the major species with a wide distribution in Iran, Iraq and Oman [19]. In fact, the impact of bacteria on cellulose degradation in the termite gut has always been a matter of debates. Early studies showed that no cellulose-degrading bacteria were present among the dominant termite species, using both aerobic and anaerobic techniques, but novel studies resulted in isolation of cellulose-degrading bacteria from a number of termite species and it seems that each termite species hosts different bacterial species [20, 21]. Due to the diversity of termite species, each species (e.g. *Microcerotermes diversus* Silvestri) needs to be separately studied.

Beside strict anaerobes, aerobic and facultatively anaerobic microbes also occur in the termite gut [7]. The results of the present survey show that there are different aerobic, facultatively anaerobic or microaerophilic cellulolytic bacteria present in the gut of *Microcerotermes diversus* (Silvestri). Strictly anaerobic cellulolytic bacteria were not investigated in this study.

A few cellulose-degrading bacteria such as *Streptomyces*, *Pseudomonas, Acinetobacter, Ochrobactrum*, *Clostridium*, *Cellulomonas*, *Bacillus*, *Paenibacillus*, *Brevibacillus*, *Eubacterium*, *Serratia*, *Citrobacter*, and *Klebsiella* have been isolated and identified from some termite species [22]. Cellulolytic bacteria identified in our study belong to different bacterial families. L2 and L12 are identified as the new genera of *Entrobacteriaceae* family. Cellulase activity has been shown in some members of *Entrobacteriaceae* family, such as *Erwinia carotovora* [23]. The cellulolytic activity of *Enterobacteriaceae* family in other termite species has occasionally been reported [24].

L9B was found to belong to *Acinetobacteriaceae*. Cellulase production has been shown for several members of *Acinetobacteriaceae* family; cellulolytic *Acinetobacteriaceae* have been isolated from termite species [24].

As the results of the phylogenetic analysis indicated, L8A closely clustered with *Pseudomonas putida*. Some members of the genus *Pseudomonas*, such as *P. fluorescens,* have been reported to produce cellulase enzymes [25].

Isolate B3 clustered with the members of the genus *Staphylococcus*. Cellulase activity is not a property commonly found in Staphylococci. However, cellulolytic staphylococci have been occasionally isolated from termites [26].

Bacillaceae family has many cellulolytic members [27], and B5B and B2A isolates belong to it. Other studies also frequently reported the isolation of cellulolytic bacilli from termites [24]. According to Wenzel et al. (2002) main portion of the cellulolytic strains from the gut of the termite *Zootermopsis angusticollis* is assigned to the genera *Bacillus* and *Paenibacillus* and the *Flexibacter* group [8], and according to Taechapoempol et al. (2011) all the effective cellulase-producing bacteria isolated from Thai higher termites of genus *Microcerotermes* were identified *Bacillus* sp. by the 16S rRNA gene sequencing method [28].

In our study, the high similarity of B5B and B2A, as well as L2 and L12, in the phylogenetic tree, may reflect the numerical abundance of *Bacillaceae* and *Entrobacteriaceae* family in the *Microcerotermes diversus* (Silvestri). CMCase activity of B5B isolate is higher than that of *Bacillus* sp. isolated from sago pith waste by Apun et al. (2000) [10] and of *Bacillus pumilus* EB3 isolated by Ariffin et al. (2006) [11].

Generally, microbial cellulases from mesophilic sources have been found to have temeperature optima of about 35–45°C and optimum pH of 4.0–8.0 [29]. Comparison of the characteristics of CMCase from B5B isolate reveals that the CMCase has an advantage over other fungal cellulases. Higher optimum pH and temperature can lead to a higher reaction rate. Cellulase from *Bacillus* B5B was found to be stable in the range of acidic pH at 60°C. These properties suggest that the *Bacillus* B5B has the potential to produce highly stable cellulase enzyme which might be used as key enzyme in the production of bioethanol from cellulose.

Newly isolated *Acinetobacter* L9B hydrolyses carboxymethyl cellulose faster than *Acinetobacter anitratus* that was isolated by Ekperigin (2007) [30], from the haemolymph of the giant African snail, *Archachatina marginata*. *Acinetobacter* L9B has the potential for cellulase production and is promising in view of use in the future.

ACKNOWLEDGMENTS

This work was supported by research vice chancellor of Shahid Chamran University of Ahwaz, Iran, project no. 94-02-04253a.

REFERENCES

1. Bhat, M.K., Cellulases and Related Enzymes in Biotechnology, *Biotechnol. Adv.* 2000, vol. 18, pp. 355–383.
2. Gao, J., Weng, H., Zhu, D., Yuan, M., Guan, F., and Xi, Y., Production and Characterization of Cellulolytic Enzymes from the Thermoacidophilic Fungal *Aspergillus terreus* M11 under Solid-State Cultivation of Corn

Stover, *Bioresour. Technol.*, 2008, vol. 99, pp. 7623–7629.

3. Kotchoni, S.O. and Shonukan, O.O., Regualtory Mutations Affecting the Synthesis of Cellulase, *World J. Microbiol. Biotechnol.*, 2002, vol. 18, pp. 487–491.
4. Nakamura, K. and Kppamura, K., Isolation and Identification of Crystalline Cellulose Hydrolyzing Bacterium and its Enzymatic Properties, *J. Ferment. Technol.*, 1982, vol. 60, pp. 343–348.
5. Maki, M., Leung, K.T., and Qin, W., The Prospects of Cellulase-Producing Bacteria for the Bioconversion of Lignocellulosic Biomass, *Int. J. Biol. Sci.*, 2009, vol. 5, pp. 500–516.
6. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K., and Stackebrandt, E., *The Prokaryotes: A Handbook on the Biology of Bacteria*, New York: Springer, 2006, 3rd ed., vol. 5, pp. 163–200.
7. Brune, A., Symbiotic Associations between Termites and Prokaryotes, *The Prokaryote*s, 3rd ed., New York: Springer, 2006, vol. 1, pp. 439–474.
8. Wenzel, M., Schönig, I., Berchtold, M., Kämpfer, P., and König, H., Aerobic and Facultatively Anaerobic Cellulolytic Bacteria from the Gut of the Termite *Zootermopsis angusticollis*, *J. Appl. Microbiol.*, 2002, vol. 92, pp. 32–40.
9. Tokuda, G. and Watanabe, H., Hidden Cellulases in Termites: Revision of an Old Hypothesis, *Biol. Lett.*, 2007, vol. 3, pp. 336–339.
10. Apun, K., Jong, B.C., and Salleh, M.A., Screening and Isolation of a Cellulolytic and Amylolytic *Bacillus* from Sago Pith Waste, *J. Gen. Appl. Microbiol.*, 2000, vol. 46, pp. 263–267.
11. Ariffin, H., Abdullah, N., Umi Kalsom, M.S., Shirai, Y., and Hassan, M.A., Production and Characterisation of Cellulase by *Bacillus pumilus* EB3, *Int. J. Eng. Technol.*, 2006, vol. 3, pp. 47–53.
12. Miller, G.C., Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, *Anal. Chem.*, 1959, vol. 31, pp. 426–428.
13. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and Williams, S.T., *Bergey's Manual of Determinative Bacteriology*, Baltimore, Maryland, USA: The Williams & Wilkins Co., 1994, 9th ed.
14. Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J., 16S Ribosomal DNA Amplification for Phylogenetic Study, *J. Bacteriol.*, 1991, vol. 173, pp. 697–703.
15. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G., The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools, *Nucleic Acids Res.*, 1997, vol. 15, pp. 4876–4882.
16. Tamura, K., Dudley, J., Nei, M., and Kumar, S., MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.*, 2007, vol. 24, pp. 1596–1599.
17. Viikari, L., Alapuranen, M., Puranen, T., Vehmaanperä, J., and Sika-aho, M., Thermostable Enzymes in Lignocellulose Hydrolysis, *Adv. Biochem. Eng. Biotechnol.*, 2007, vol. 108, pp. 121–145.
18. Habibpour, B., Ekhtelat, M., Khocheili, F., and Mossadegh, M.S., Foraging Population and Territory Estimates for *Microcerotermes diversus* (*Isoptera: Termitidae*) through Mark-Release-Recapture in Ahwaz (Khouzestan, Iran), *J. Econ. Entomol.*, 2010, vol. 103, pp. 2112–2117.
19. Edwards, R. and Mill, A.E., *Termites in Buildings—Their Biology and Control,* East Grinstead, UK: Rentokil Ltd., 1986.
20. Adams, L. and Boopathy, R., Isolation and Characterization of Enteric Bacteria from the Hindgut of Formosan Termite, *Bioresour. Technol.*, 2005, vol. 96, pp. 1592–1598.
21. Graber, J.R. and Breznak, J., Physiology and Nutrition of *Treponema primitia*, an H_2/CO_2-Acetogenic Spirochete from Termite Hindguts, *Appl. Environ. Microbiol.*, 2004, vol. 70, pp. 1307–1314.
22. Schäfer, A., Konrad, R., Kuhnigk, T., Kämpfer, P., Hertel, H., and König, H., Hemicellulose-Degrading Bacteria and Yeasts from the Termite Gut, *J. Appl. Microbiol.*, 1996. vol. 80, pp. 471–478.
23. Chatterjee, A., Cui, Y., Liu, Y., Dumenyo, C.K., and Chatterjee, A.K., Inactivation of *rsmA* Leads to Overproduction of Extracellular Pectinases, Cellulases, and Proteases in *Erwinia carotovora* subsp. *carotovora* in the Absence of the Starvation/Cell Density-Sensing Signal, N-(3-Oxohexanoyl)-L-Homoserine Lactone, *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 1959–1967.
24. Ramin, M., Alimon, A.R., and Abdullah, N., Identification of Cellulolytic Bacteria Isolated from the Termite *Coptotermes curvignathus* (Holmgren), *J. Rapid. Methods Autom. Microbiol.*, 2009, vol. 17, pp. 103–116.
25. Hall, J., Black, G.W., Ferreira, L.M.A., Millward-Sadler, S.J., Ali, B.R.S., Hazlewood, G.P., and Gilbert, H.J., The Non-Catalytic Cellulose-Binding Domain of a Novel Cellulase from *Pseudomonas fluorescens* subsp. *cellulosa* is Important for the Efficient Hydrolysis of Avicel, *Biochem. J.*, 1995, vol. 309, pp. 749–756.
26. Sarkar, A., Varma, A., and Sarkar, A., Influence of Cellulolytic Organisms Associated with a Termite, *Odontotermes obesus*, on Carbon Mobility in a Semiarid Ecosystem, *Arid Soil Res. Rehabil.*, 1988, vol. 2, pp. 75–84.
27. Ito, S., Alkaline Cellulases from Alkaphilic *Bacillus*: Enzymatic Properties, Genetics, and Application to Detergents, *Extremophiles*, 1997, vol. 1, pp. 61–66.
28. Taechapoempol, K., Sreethawong, T., Rangsunvigit, P., Namprohm, W., Thamprajamchit, B., Rengpipat, S., and Chavadej, S., Cellulase-Producing Bacteria from Thai Higher Termites, *Microcerotermes* sp.: Enzymatic Activities and Ionic Liquid Tolerance, *Appl. Biochem. Biotechnol.*, 2011, vol. 164, pp. 204–219.
29. Dutta, T., Sahoo, R., Sengupta, R., Ray, S.S., Bhattacharjee, A., and Ghosh, S., Novel Cellulases from an Extremophilic Filamentous Fungi *Penicillium citrinum*: Production and Characterization, *J. Ind. Microbiol. Biotechnol.*, 2008, vol. 35, pp. 275–282.
30. Ekperigin, M.M., Preliminary Studies of Cellulase Production by *Acinetobacter anitratus* and *Branhamella* sp., *Afr. J. Biotechnol.*, 2007, vol. 6, pp. 28–33.