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Bacterial strains with cellulolytic and xylanolytic activity originated from different isolation sources

Éva Laslo, Mihály Koncz, Szabolcs Toró, Szabolcs Lányi, Beáta Ábrahám

Sapientia Hungarian University of Transylvania, Faculty of Economics, Socio-Human Sciences and Engineering, Department of Bioengineering Libertăţii Sq. Nr. 1, 530104 Miercurea Ciuc, Romania.
Corresponding author email: lasloeva@yahoo.com.

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A B S T R A C T

Structural polysaccharide degrading bacteria can potentially be used in different biotechnological processes for the initiation of enzymatic decay in plant biomass. These cellulolytic and xylanolytic bacteria represent valuable sources of hydrolytic enzymes. Our aim was to isolate and characterize bacterial strains, which contribute to the increase of water soluble carbohydrate content in different raw materials of plant biomass. Due to their beneficial effect, these bacteria can improve the hydrolysis rate of lignocellulose biomass. During our work, a number of 208 bacterial isolates with cellulose and hemicellulose-degrading capacity have been obtained. The selected strains have been assayed for cellulolytic and xylanolytic activities. 47 isolates with structural polysaccharide degrading capacity have been identified based on their16S ribosomal DNA gene sequence analysis. The identified bacterial strains belong to different genera such as: Bacillus, Stenotrophomonas, Acinetobacter, Paenibacillus, Comamonas, Corynebacterium, Flavobacterium, Janthinobacterium, Kurthia, Leucobacter, Pseudomonas, and Sphingobacterium.

Key words: *cellulose, , cellulolytic bacteria, xylan, 16SrDNA, xylanolytic bacteria*

Introduction

Plant biomass represents a common and valuable natural resource for many industries. Due to their enzymes, microorganisms play an important role in the treatment of plant biomass. They also contribute to cost-effective and gentle technological steps in biomass processing. The main compounds resulting from the breakdown of hemicellulose and xylan are carbohydrates (Sizova et al.,2011, Okeke and Lu, 2011). The major structural element of all vegetal cell walls is lignocellulose which represents a renewable organic material. This consists of 30% cellulose, 30% hemicellulose, 35% pectin, while the remaining 5% are proteins and phenol derivatives(Ochoa-Villarreal and Aispuro-Hernández, 2012). Due to the action of cellulases produced by different microorganisms, the release of carbohydrates from cellulose can be biologically achieved (Mood et al., 2013, John et al., 2011). However, total enzymatic breakdown of cellulose is the result of a synergetic effect of several enzymes, such as endoglucanases, exoglucanases, cellobiases and β-glucosidases.

Due to the pronounced orientation towards renewable energy, there has been an increased need for efficient microorganisms from different ecosystems that hydrolyze cellulose, contributing to the breakdown of lignocellulosic biomass. Cellulolytic bacteria are diverse and occur in different environments such as compost, soil, wastewater, livestock gut and feces (Schwarz, 2001, Rakotoarivonina et al., 2012).

Hemicellulose, on the other hand, has a more complex structure. The conversion of xylan plays a key role in the degradation of plant cell wall (Shallom and Shoham, 2003.). Different enzymes are involved in the biodegradation of xylan-based structures, like endo-β (1-4) xylanases, β(1-4) xylosidases, α-L-arabinofuranosidase, α-glucuronosidase, as well as acetylxylan esterase (Saha 2003).

The aim of this study was to isolate and characterize microorganisms originating from different environmental niches which contribute to the increase of water soluble carbohydrate content in lignocellulose biomass, enhancing its conversion and availability in the agro-industrial carbon flow and nutrient cycle.

Materials and methods

Isolation of cellulolytic and xylanolytic bacteria

Bacterial strains with cellulose and xylan hydrolyzing capacities have been isolated from different ecological habitats, such as the following: pine forest soil, deciduous forest soil, meadow soil with hay, rumen, ruminant (goat) feces, mushroom substrate, mature compost, brewery sludge, bark beetle feces, the surface of mushrooms, as well as corn and legumes (leguminous) silage. Isolation has been carried out by direct inoculation method and enrichment technique (Gupta et al., 2012, Castro et al., 2014) as follows: 1 g of each substrate has been dissolved in 9 ml sterile physiological solution and serial dilutions have been prepared. 0.1 ml from these have been spread on the surface of Bushnell Haas agar medium (BHM: 1.0 g/L NH_4NO_3, 1.0g/L KH_2PO_4, 0.5g/L $MgSO_47H_2O$; 1.0 g/L yeast extract) supplemented with one of the carbon sources: 15g/L CMC (carboxymthyl celullose), xylan or cellulose and containing Congo Red dye (Huan et al., 2009, Teather and Wood, 1982). The inoculated dishes have been incubated for 7 days at room temperature. Isolation from the surface of the media has been performed according to the size of the halo zone, developed around the bacterial colonies. Distinctive purified colonies have been inoculated on Nutrient agar slants and stored at 4 °C.

For the enrichment technique, Bushnell Haas medium has been used, supplemented with different carbon sources CMC, DDGS (distiller's dried grains with solubles) and alfalfa) (Lo et al., 2011, Techapun et al., 2002). These mediums have been inoculated with 1 g from each of the 12 substrates. Cultures have been incubated at room temperature for a period of 7 days. Potential cellulose and xylan-degrading bacteria have been inoculated on Congo Red containing BHM medium. Isolation from the surface of these media has been performed as described previously.

Determination of cellulolytic and xylanolytic capacity of the bacterial isolates

For the determination of cellulolytic and xylanolytic capacity of the bacterial isolates, the 3, 5-dinitrosalicylic acid (DNSA) method has been used. 49 bacterial isolates presenting different colony morphology have been selected. These isolates have been grown in liquid Nutrient medium for 24 hours at 28°C, 150 rpm. 20 ml of liquid BHM medium supplemented with different carbon sources (cellulose, xylan, CMC) has been inoculated with 2% of bacterial suspension and has been incubated for 7 days.

The amount of reducing sugars generated by the cellulolytic or xylanolytic activities has been determined after three, five and seven days of incubation. 1 ml of bacterial suspension has been centrifuged at 10000 rpm, 4 °C and the obtained supernatant has been further analyzed for the reducing sugar content (Miller 1959, Bailey 1988, Zhao et al., 2008).

For the selected strains, the cellulase and xylanase enzyme activity has been determined using a glucose and xylose calibration curve.

Identification of cellulolytic and xylanolytic bacterial strains

47 bacterial isolates have been identified based on 16S rDNA sequence analysis. Genomic DNA has been isolated using an AccuPrep Genomic DNA Isolation Kit from Bioneer, according to the manufacturer's protocol. A part of the bacterial 16S rDNA gene has been amplified with the universal oligonucleotides 27f 5' AGAGTTTGATCMTGGCTCAG 3'and 1492r 5'TACGGYTACCTTGTTACGACTT3'.

The nucleotide sequence determination has been performed by LGC Genomics. Multiple sequence alignments and manual corrections have been performed with Chromas (Technelysium Pty. Ltd., South Brisbane, Australia). Phylogenetic analyses have been conducted using the Molecular Evolutionary Genetics Analysis 4 system (www.megasoftware.net) (Tamura et al., 2007). The isolates have been identified through comparison of the sequences using the EzTaxon server (www.ezbiocloud.net/eztaxon) on the basis of 16S rDNA sequence data (Kim et al., 2012).

Phenotypic and biochemical characterization of the selected strains

Biochemical characterization of the most promising 20 bacterial isolates has been carried out using Biolog GENIII MicroPlates, according to the manufacturer's protocol, containing 71 carbon source utilization assays and 23 chemical sensitivity assays.

Results and discussion

Structural polysaccharide degrading bacteria have a pivotal role in the degradation of cellulose and hemicellulose materials (Nyonzo et al., 2014). There is a higher need for these microorganisms that break down plant lignocellulose materials which represent one of the most renewable organic resources. Earlier studies reported on the high diversity of bacterial strains with cellulose degrading capacity that has biotechnological applicability (Ghio et al.,2012, Bashir et al.,2013). A total number of 208 bacterial strains have been isolated from different environmental samples based on their ability to grow on cellulose, CMC or xylan, as the sole carbon source.

Based on the high halo zone developed around the colonies, a number of 74 bacterial isolates have been selected and analyzed for their polysaccharide degrading capacity, along with determination of their phenotypic patterns. The origin of these bacterial isolates is different. Eight of them have been obtained from deciduous forest soil, two from bark beetle feces, 14

from silages, 15 from the rumen of cows, four from industrial brewery sludge, eight from mature compost, four from meadow soil, three from pine forest soil, seven from Pleurotus ostreatus substrate, three from mushrooms, and six from goat feces.

47 isolates have been identified based on 16S ribosomal DNA gene sequence analysis. The identified beneficial bacterial isolates belong to 4 phylum: Proteobacteria (42%), Firmicutes (29%), as well as Actinobacteria and Bacteroidetes (29%). The phylogenetic relationship of the structural polysaccharide degrading bacteria originated from different environments is illustrated on the phylogenetic tree (Figure 1.), constructed using the Mega 4 software tool.

From the assayed bacterial strains, 24 (53%) have released high content of reducing sugars from structural polysaccharides. The most representative bacterial strains are: Acinetobacter beijerinckii K121, Bacillus safensis K193, Janthinobacterium agaricidamnosum C101, Janthinobacterium svalbardensis K140, Kurthia massiliensis C31, Paenibacillus odorifer K183, Paenibacillus peoriae K02A, Pseudomonas geniculata K09, Pseudomonas japonica K175, Raoultella planticola C107, Sphingobacterium kitahiroshimense K185, Stenotrophomonas rhizophila K142, Stenotrophomonas rhizophila K143.

Figure 1: Phylogenetic diversity of the identified cellulolytic and xylanolytic bacteria originated from different environments

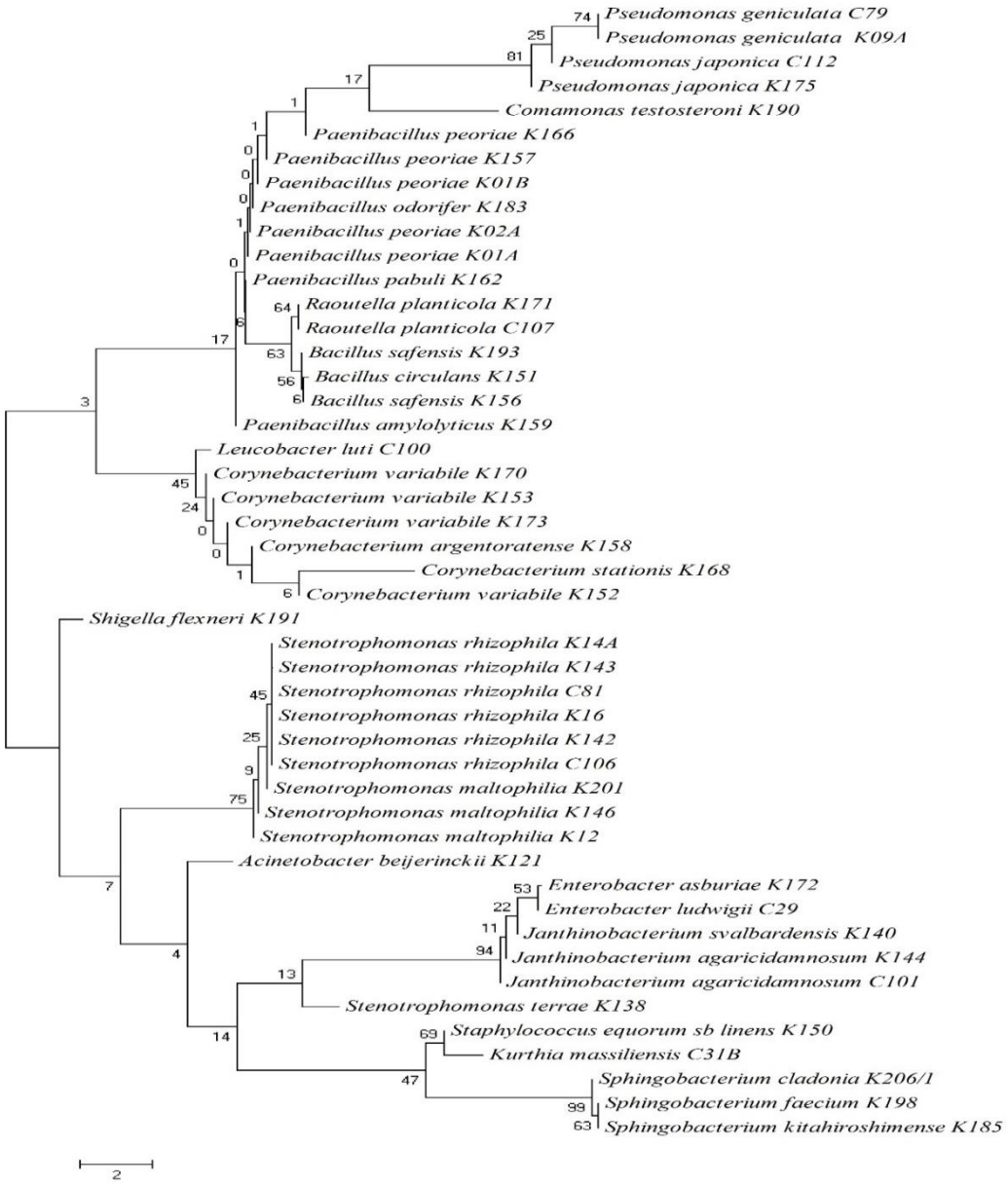

Figure 1: Phylogenetic diversity of the identified cellulolytic and xylanolytic bacteria originated from different environments

Enzyme activities, which resulted from the sugar release, have been lower on the third day, probably due to the prolonged adaptation of the bacterial isolates to the environment. The lowest percentage has been achieved on the seventh day, resulting probably from the product inhibition effect and carbon source depletion.

On the fifth day 32 (71 %) of the tested bacterial isolates could degrade xylan, and 20 (44 %) of the tested bacterial isolates degraded CMC resulting in reducing sugars. From the assayed three structural polysaccharides, these two are considered to be the most favorable.

Paenibacillus peoriae K02A, Pseudomonas geniculata K09, Bacillus safensis K193 could degrade all three polysaccharides according to the results of the DNSA method on the fifth day. Raoultella planticola C107, Stenotrophomonas rhizophila K143, Stenotrophomonas rhizophila K14A, Pseudomonas japonica K175, Bacillus safensis K193, Sphingobacterium cladonia K206/1 have shown polysaccharide degrading activity in each case of the tested polysaccharides on the seventh day of the incubation.

In the case of xylan degradation, only six of the bacterial isolates showed no or very weak xylan degrading activity on the fifth and seventh days.

Acinetobacter beijerinckii K121, Janthinobacterium agaricidamnosum K144, Janthinobacterium svalbardensis K140, Pseudomonas japonica C112, Shigella flexneri K191, Stenotrophomonas terrae K138 bacterial strains have shown a high xylan degrading capacity.

Two bacterial strains, Bacillus safensis K193 and Stenotrophomonas rhizophila K16 could hydrolyze all three assayed polysaccharides on the third day of the incubation. Acinetobacter beijerinckii K121, Janthinobacterium agaricidamnosum C101, Janthinobacterium svalbardensis K140 and Stenotrophomonas rhizophila K142 have shown cellulose and CMC degrading activity on the fifth day, whereas three of the strains, Bacillus safensis K193, Paenibacillus peoriae K02A and Pseudomonas geniculata K09 could degrade all the three polysaccharides tested. Bacillus safensis K193, Pseudomonas japonica K175, Raoultella planticola C107, Sphingobacterium cladonia K206/1, Stenotrophomonas rhizophila K143 and Stenotrophomonas rhizophila K14A could degrade all the three polysaccharides on each of the last two days.

Among the assayed bacterial isolates only some have shown promising results. In the case of the strain Paenibacillus peoriae K01B isolated from corn silage, the activity of the endo-cellulases was 1.1975 UI on the third day of the incubation and 1.598 UI on the fifth day. The highest exo-cellulase activity has been observed in the case of the strain Pseudomonas japonica K175 isolated from deciduous forest soil. The reducing sugar amount liberated during one minute was 1.927 µmol on the fifth day. Jeong et al. 2012, determined some genes that are responsible for the utilization of plant derived polysaccharides in the case of plant beneficial soil bacteria Paenibacillus peoriae.

It is to be noticed, that the strain Stenotrophomonas rhizophila K143 has shown activity on CMC, although it has been isolated from mature compost and grown on cellulose powder. This strain showed 0.9474 UI on the third day of the incubation. Regarding strains of Stenotrophomonas sp., Raj et al., 2013, showed that Stenotrophomonas maltophilia produced highly thermostable xylanase.

The highest amount of xylanolytic enzymes were produced by Bacillus safensis K193 on the fifth day after incubation, when the enzyme activity value reached 6.7447 UI. This strain has been isolated from silage sample. In the case of Pseudomonas japonica K175, the enzyme activity was 0.5758 UI on the third day and 0.6267 UI on the fifth day. Raoultella planticola C107 has shown 0.9131 UI, while the strain Paenibacillus peoriae K02A has reached a value of 0.5 UI. The results have pointed out that most of the bacterial isolates analyzed in this study utilize xylan well.

Our findings on Bacillus safensis being a promising strain are supported by Lateef. Et al., 2015. They reported that the safe industrial microorganism has unique abilities. This bacterium produces various industrial enzymes and industrially applicable secondary metabolites.

Metabolic patterns of the most promising 20 bacterial strains have been determined using Microlog GenIII Biolog microplates. The analyzed bacterial strains differ in fermentation patterns and chemical sensitivity. Eight (40%) from the 20 analyzed strains have shown pectin utilization capability, nine (45%) strains can utilize Tween 40 as a substrate, twelve (60%) can utilize D-cellobiose and gelatin, five of the strains (25%) degrade myo-inositol, seven (35%) utilize inosine and also seven (35%) utilize raffinose as a substrate.

Sphingobacterium faecium K198 degrades all of the following five substrates: pectin, Tween 40, D-cellobiose, gelatine, raffinose and cellobiose. Pectin and gelatin are utilized by Bacillus safensis K193, Janthinobacterium agaricidamnosum C101, Sphingobacterium cladonia K206/1 and Sphingobacterium faecium K198. Regarding the chemical sensitivity of the analyzed bacteria, eighteen (90%) from the 20 bacteria grow on medium containing 1% NaCl, ten of the tested bacteria (50%) grow on 4% NaCl, but only four strains (20%) Bacillus safensis K193, Janthinobacterium agaricidamnosum C101, Leucobacter luti C100, Raoultella planticola C107) can survive in a media containing 8% NaCl. According to the analyzed antibiotic susceptibility of the tested bacteria, 50% present sensitivity to troleandomycin, twelve (60%) to rifamycin SV, ten (50%) to lincomycin, five (25%) to nalidixic acid and nine (45%) to vancomycin. According to the biochemical characterization performed with the Biolog system, Leucobacter luti C100 not only degrades structural

polysaccharides, but can also survive in a media containing 8% of NaCl, and has shown resistance to troleandomycin and rifamycin, while the strains Comamonas testosteroni K190, Pseudomonas geniculata K09A, Corynebacterium argentoratense K158, Corynebacterium stationis K168 have shown resistance to troleandomycin, rifamycin and lincomycin.

Not only have the assayed bacterial isolates shown highest cellulase and xylanase activities, but based on the metabolic pattern results, some of the bacteria can utilize other plant derived polysaccharides and have metabolic activity in the presence of different chemicals. These findings suggest that bacterial strains can play a significant role in biodegradation processes.

Conclusions

The present study highlights the diverse community of aerobic and facultative anaerobic cellulolytic and xylanolytic bacteria in different environmental samples. Some of these bacterial strains have shown potential to be used in different biotechnological processes due to the degradation capacity of structural polysaccharides resulting in water soluble sugars.

The results revealed that Bacillus safensis K193 and Stenotrophomonas rhizophila K16 have a major contribution in the degradation of the assayed lignocellulosic materials.

A microbial consortium based on the assayed and selected strains is expected to increase the yield of the enzymatic decay of cellulolytic and hemicellulolytic plant materials.

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References

Bailey MJ. 1988. A note on the use of dinitrosalicylic acid for determining the products of enzymatic reactions. Appl Microbiol Biotechnol. 1988; 29: 494-496.

Bashir Z, Kondapalli VK, Adlakha N, Sharma A, Bhatnagar RK, Chandel G, Yazdani SS.2013. Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts. SCI REP. 2013; 3:2558.

Castro RA, Quecine MC, Lacava PT, Batista BD, Luvizotto DM, Marcon J, Azevedo JL. 2014. Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. SpringerPlus. 3: 382-391.

Ghio SG, Di Lorenzo S, Lia V, Talia, Cataldi A, Grasso D, Campos E. 2012. Isolation of Paenibacillus sp. and Variovorax sp. strains from decaying woods and characterization of their potential for cellulose deconstruction. Int J Biochem Mol Biol.3:352-364.

Gupta P, Samant K, Sahu A. 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Int J Microbiol. 2012:1-5.

Huan W, Li X, Zhou Y, Ma Q, Chen Y. 2009. Simultaneous cloning and expression of two cellulase genes from Bacillus subtilis newly isolated from Golden Takin (Budorcastaxicolor Bedfordi). Biochem Biophys Res Commun. 383: 397-400.

Jeong H, Choi S-K , Park S-Y, Kim S H, Parka S-H. Draft Genome Sequence of Paenibacillus peoriae Strain KCTC 3763T.2012. J. Bacteriol. 2012;194:1237-1238.

John RP, Anisha GS, Nampoothiri KM, Pandey A. 2011. Micro and macroalgal biomass: a renewable source for bioethanol. Bioresource Technol. 102:186-193.

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H.2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species Int J Syst Evol Microbiol. 62:716-721.

Lateef A, Adelere IA, Gueguim-Kana E-B.2015. The biology and potential biotechnological applications of Bacillus safensis. Biologia.70: 411-419.

Lo W, Lu C, Chen CY, Chen WM, Chang JS. 2011. Characterization and high-level production of xylanase from an indigenous cellulolytic bacterium Acinetobacter junii F6-02 from southern Taiwan soil. BIOCHEM ENG J.53: 77-84.

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

Mood S, Golfeshan HAH, Tabatabaei M, Jouzani GS, Najafi GH, Gholami M, Ardjmand M. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew Sust Energ Rev. 27:77-93.

Nyonzo T, Shinkai T, Mitsumori M. 2014.Improved culturability of cellulolytic rumen bacteria and phylogenetic diversity of culturable cellulolytic and xylanolytic bacteria newly isolated from the bovine rumen. FEMS Microbiol Ecol. 88:528-537.

Ochoa-Villarreal M, Aispuro-Hernández E. 2012. Plant cell wall polymers: function, structure and biological activity of their derivatives, In: Gomes A De S. Polimerization, INTECH Open Access Publisher-

Okeke BC, Lu J. 2011. Characterization of a Defined Cellulolyticand Xylanolytic Bacterial Consortium for Bioprocessing of Cellulose and Hemicelluloses. Appl Biochem Biotechnol.163: 869–881.

Raj A, Kumar S, Singh K. 2013. A Highly Thermostable Xylanase from Stenotrophomonas maltophilia: Purification and Partial Characterization. Enzyme Res. 2013:1-8.

Rakotoarivonina H, Hermant B, Monthe N, Rémond C. 2012. The hemicellulolytic enzyme arsenal of Thermobacillus xylanilyticus depends on the composition of biomass used for growth. Microb Cell Fact. 11:159-161.

Saha BC.2003. Hemicellulose bioconversion. JIMB.30: 279-291.

Schwarz WH. 2001. The cellulosome and cellulose degradation by anaerobic bacteria. Appl. Microbiol. Biotechnol. 56:634-649.

Shallom D, Shoham Y.2003. Microbial hemicellulases. Curr Opin Microbiol. 6:219–228.

Sizova MV, Izquierdo JA, Panikov NS, Lynd LR. 2011. Cellulose-and xylan-degrading thermophilic anaerobic bacteria from biocompost. Appl Environ Microbiol. 77: 2282-2291.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol. Biol. Evol. 24:1596–1599.

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

Techapun C, Charoenrat T, Watanabe M, Sasaki K, Poosaran N. 2002 Optimization of thermostable and alkaline-tolerant cellulase-free xylanase production from agricultural waste by thermotolerant Streptomyces sp., Ab106, using the central composite experimental design. Biochem Eng J. 99-105.

Zhao K, Xue PJ, Gu GY. 2008. Study on Determination of Reducing Sugar Content Using 3, 5- Dinitrosalicylic Acid Method. Food Science. 29: 534-536.