Tanzania Journal of Science 47(1): 204-213, 2021
ISSN 0856-1761, e-ISSN 2507-7961
© College of Natural and Applied Sciences, University of Dar es Salaam, 2021

Cellulase-Producing Bacteria Isolated from Mufindi Paper Mill Industrial Effluent, Iringa Tanzania

Erick M. Sinza[1], Aneth D. Mwakilili[1], Cyprian B. Mpinda[1*] and Sylvester L. Lyantagaye[1,2]
[1]*Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences, University of Dar es Salaam, P. O. Box 35179, Dar es Salaam, Tanzania.*
[2]*Department of Biochemistry and Pharmacology, Mbeya College of Health and Allied Sciences, University of Dar es Salaam, P. O. Box 608, Mbeya, Tanzania.*
**Corresponding author e-mail: cyprianmpinda@yahoo.com*
Co-authors' e-mails: ericksinza@gmail.com (Sinza); anethdavid367@gmail.com (Mwakilili); lyantagaye@gmail.com (Lyantagaye)
Received 6 Aug 2020, Revised 11 Dec 2020, Accepted 28 Dec 2020, Published Feb 2021
https://dx.doi.org/10.4314/tjs.v47i1.18

Abstract

Effluent and effluent-contaminated soil samples collected from Mufindi Paper Mill Limited, Iringa Tanzania were investigated for the presence of cellulolytic bacteria. The components of cellulase are endoglucanase, exoglucanase and β-glucosidase and these are very important for complete hydrolysis of cellulose to glucose monomers. Isolates were grown on nutrient agar and screened for cellulolytic activity on carboxymethylcellulose (CMC) agar plates using Gram's iodine solution. Cellulases activities; endoglucanase, exoglucanase and β-glucosidase were assayed using CMC, avicel and cellobiose substrates, respectively by dinitrosalicyclic acid method. Out of 17 cellulolytic isolates obtained, crude enzyme extracts of only five isolates expressed all cellulases activities, the highest activity being on cellobiose followed by CMC and least on avicel. The maximum endoglucanase (0.0701 ± 0.010 U/ml), exoglucanase (0.0081 ± 0.001 U/ml) and β-glucosidase (0.0738 ± 0.002 U/ml) activities from submerged fermentation culture were observed from isolate 8, 1 and 12, respectively. Based on 16S rRNA sequences analyses, three isolates were identified as *Pararheinheimera mesophila, Bacillus cereus* and *Bacillus flexus*. The other two isolates were identified as *Bacillus megaterium*.

Keywords: Cellulose, Endoglucanase, Exoglucanase, β-glucosidase, 16S rRNA.

Introduction

Lignocellulosic biomasses are the enormous renewable, cost effective and useful raw materials for industrial production of biofuels, chemicals and other bio-products (Moe et al. 2012). Being abundant, lignocellulosic biomasses may be obtained from many sources such as agricultural residues, forest wastes, industrial wastes and municipal solid wastes (Zabed et al. 2016). They are made up of mainly cellulose (40–50% of dry weight), which is a linear polymer of glucose residues connected by β-1,4-glycosidic linkages; hemicellulose (25–35% of dry weight) and lignin (15–20% of dry weight) (Smith et al. 2010). Cellulose hydrolysis has been a topic of interest for many researchers for establishment of large-scale conversion of lignocellulosic biomasses into fermentable sugar (Sukumaran et al. 2005).

Cellulose can be converted into glucose monomer either through chemical hydrolysis or enzymatic hydrolysis; however, the later method is considered more advantageous as it gives high yields while utilizing less energy (Yang et al. 2011). Cellulase is the general

term for an enzyme system that consists of three components of enzymes; endoglucanases or carboxymethylcellulases (EC 3.2.1.4), exoglucanases or cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21), which work in synergistic manners for complete hydrolysis of cellulose polymer (Taha et al. 2016). Endoglucanases cut the internal β-1,4-bonds of cellulose chains randomly, thereby creating new chain ends. Exoglucanases then act on the released cellulose fragments to produce cellobiose or glucose residues from reducing and non-reducing ends. β-glucosidases finally hydrolyze cellobiose into glucose units (Zabed et al. 2016).

Currently, most studies on bioconversion of cellulose materials are based on cellulases from members of fungi *Trichoderma* particularly *T. reesei* due to their ability to secrete all necessary components of cellulase essential for complete hydrolysis of cellulose (Gusakov 2011). *T. reesei* cellulase system consists of high activity endoglucanases and exoglucanase with deficiency in β-glucosidases activity. Production of low level of β-glucosidases has resulted into accumulation of cellobiose residues during cellulose hydrolysis process that in turn inhibits cellulase activity (Singhania et al. 2013, Qian et al. 2017). Several efforts to manipulate *T. reesei* strains by genetic engineering approaches with the aim of obtaining a single strain with high levels of cellulase components have been done (Qian et al. 2017). However, the cellulolytic potential of these strains is still not sufficient towards large-scale bioconversion of cellulose biomasses (Liet al. 2018).

Studies are currently focusing on isolating microorganisms with superior cellulase activity and more emphasis is placed on cellulolytic bacteria as they show high growth rates and other potential characteristics such as possession of stable glycoside hydrolases with increased synergistic actions over fungi (Akhtar et al. 2016). However, despite the efforts of these studies, no efficient cellulase system has been reported. This study was therefore designed to isolate, screen and characterize cellulase-producing bacteria from Mufindi paper mill industrial effluent with the aim of searching for novel bacteria strains capable of producing a complete cellulase system for biotechnology applications such as production of biofuels and waste management.

Materials and Methods

Sample collection

Effluent samples were collected during November 2019 from Mufindi Paper Mill Ltd, Iringa Tanzania from three ponds; before treatment, preliminary treatment and discharged ponds. Samples were collected at a depth of about 2 cm from six randomly selected points at each pond in sterile plastic containers and placed into a cooler box. Effluent-contaminated soil near each pond was also collected. Samples were transported to the Microbiology Laboratory at the Department of Molecular Biology and Biotechnology (MBB), University of Dar es Salaam and stored at 4 °C until used.

Isolation of bacteria

Isolation of bacteria was done as described by Mahjabeen et al. (2016). All samples were serially diluted up to 10^{-6} and thereafter 100 µL of each sample from dilutions 10^{-4}, 10^{-5} and 10^{-6} were cultured by pour plating technique on nutrient agar (NA) supplemented with nystatin, an antifungal agent, at the concentration of 50 µg/mL to inhibit growth of fungi and incubated at 30 ^{o}C for 24 hours. After 24 hours of incubation period, unique well-separated colonies were selected and sub-cultured onto fresh NA plates until pure colonies were obtained.

Primary screening of cellulolytic bacteria

Screening of isolates with cellulolytic activity was done according to Naresh et al. (2019). Each isolate was inoculated in the middle of carboxymethyl cellulose (CMC) agar plates (CMC agar composition (g/L): KH_2PO_4 1.36 g, $MgSO_4.7H_2O$ 0.20 g, NaCl 2.00 g, $(NH_4)_2SO_4$ 1.00 g, $FeSO_4.7H_2O$ 0.01 g, CMC 3.00 g, yeast

extract 1 g, agar powder 15 g, with pH adjusted to 7). The plates were then incubated for 3 days at 30 °C. In order to visualize cellulolytic activity, plates were flooded with Gram's iodine solution (2 g KI and 1g I_2 in 300 mL water) and allowed to stand for 5 minutes at room temperature. Pure cultures of *Escherichia coli* and *Bacillus subtilis* were also included as negative and positive control, respectively. Observation of a clear zone around the colony indicated cellulose utilization. Cellulolytic index of each isolate was calculated by using the equation below (Ferbiyanto et al. 2019).

$$\text{Cellulolytic Index} = \frac{\text{Diameter of zone (mm) - Diameter of bacterial colony (mm)}}{\text{Diameter of bacterial colony (mm)}}$$

Production of cellulases enzyme

Isolates that formed halo zone on CMC agar plates together with *E.coli* and *B. subtilis* were selected for quantitative determination of cellulases activities in submerged fermentation as described by Arimurti et al. (2017). One loop of each of selected bacteria isolate was transferred from NA agar plates and inoculated into 10 mL nutrient broth in 50 mL Erlenmeyer flask and incubated at 30 °C on a rotary shaker (Edmund Buhler, 7400 Tubingen Shaker-SM 25, Germany) at 120 rpm for 24 hours. Afterwards, 5 mL of each bacterial inoculum was transferred into 100 mL fermentation broth with composition similar to the isolation medium in 250 mL Erlenmeyer flasks. The flasks were then incubated at 30 °C on the above mentioned rotary shaker at 120 rpm for 72 hours. After 72 hours incubation, fermented broth was centrifuged at 12000 rpm (Microcentrifuge 5417R, Eppendorf, Germany) for 10 minutes at 4 °C. The supernatant of each broth was used as crude enzyme extract for determination of enzyme activity.

Enzyme Assays

Determination of endoglucanase activity

Endoglucanase activity was measured according to the method described by Zhang et al. (2009). The reaction mixture consisting of 0.5 mL of substrate solution (2% w/v CMC prepared in 0.05 M citrate buffer, pH 4.8) and 0.5 mL of crude enzyme extract was incubated in a water bath (GFL D3006, Germany) at 50 °C for 30 minutes. Glucose produced from CMC breakdown was quantified by dinitrosalicylic acid (DNS) method. Specifically, 3 mL of DNS reagent was added to the reaction mixture and the resulting mixture was boiled at 100 °C (GFL D3006, Germany) for 5 minutes. Two blanks (substrate blank and enzyme blank) were also included. The test tubes were cooled for 5 minutes in an ice bath and absorbance at 540 nm was measured using a UV-Visible spectrophotometer (UV-1800 spectrophotometer, Shimadzu, Japan). A standard glucose curve was used to calculate the amount of glucose released. Enzyme activity was determined as described by Touijer et al. (2019).

$$\text{Enzyme activity (U/mL)} = \frac{\text{Glucose released (mg)} \times 1000}{180.156 \times \text{Incubation time (min)} \times \text{Enzyme volume used (mL)}}$$

Where, 180.156 = molecular weight of glucose (g/mol).

In all assays, one enzyme unit (1U) was defined as the amount of enzyme producing one µmol of reducing sugar measured as glucose per minute under standard assay conditions (Arimurti et al. 2017).

Determination of exoglucanase activity

Exoglucanase activity was determined according to the method described by Garate (2017). The reaction mixture consisting of 1.6 mL of substrate solution (1% Avicel w/v prepared in 0.05M citrate buffer, pH 4.8) and 0.4 mL of crude enzyme extract was incubated in a water bath (GFL D3006, Germany) at 50 °C for 120 minutes. The reaction mixtures were cooled in ice bath and then centrifuged at 14000 rpm (MIKRO 220, Germany) for 2 minutes at room temperature. 1 mL of supernatant was then used to determine the quantity of glucose produced by DNS method as described above.

Determination of β-glucosidase activity
β-glucosidase activity was measured by using 15 mM cellobiose prepared in 0.05 M citrate buffer (pH 4.8) according to the method described by Ochieng (2014). For this assay, the reaction mixture containing 0.5 mL of crude enzyme extract and 0.5 mL of cellobiose solution was incubated in a water bath (GFL D3006, Germany) at 50 °C for 30 minutes. The quantity of glucose produced from cellobiose hydrolysis was determined by DNS method as described above.

Molecular characterization of cellulolytic bacteria

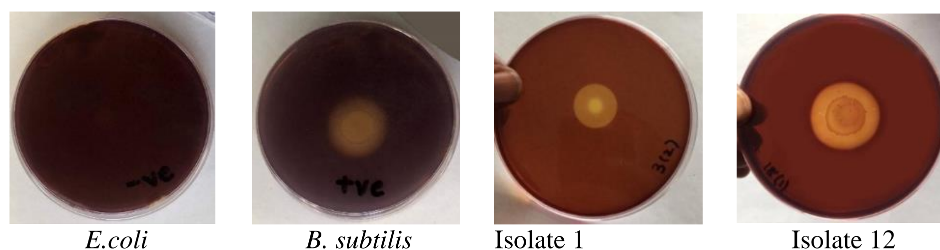
Extraction of genomic DNA
Five isolates (1, 11, 12, 14 and 15) that exhibited complete cellulase system were chosen for molecular characterization. Extraction of genomic DNA was carried out as described by Kiio et al. (2016) by using ZymoBIOMICS™ DNA Miniprep Kit according to manufacturer's instructions from bacteria cultures grown overnight in 50 mL nutrient broth at 30 °C on a rotary shaker (Edmund Buhler, 7400 Tubingen Shaker-SM 25, Germany) at 120 rpm. Concentration and purity of the extracted DNA was determined using nanodrop spectrophotometer (Nanodrop One, Thermo Fisher scientific, USA).

Polymerase chain reaction (PCR) and sequencing of 16S rRNA gene
PCR was performed using two primers: 27F (5'…AGAGTTTGATC(AC)TGGCTCAG…3') and 1492R (5'…TACGG(CT)TACCTTGTTACGACTT…3) (Kiio et al. 2016). PCR reaction used 20 µL total volume consisted of 10 µL of NEB OneTaq 2x master mix with standard buffer, 1 µL of genomic DNA, 1 µL of each of forward and reverse primers and 7µL of nuclease free water. PCR conditions were set at: 5 minutes at 94 °C for initial denaturation step followed by 35 cycles at 94 °C for 30 seconds, 50 °C for 30 seconds and 68 °C for 90 seconds, with a final extension step at 68 °C for 5 minutes. The integrity of the PCR products was visualized on a 1% agarose gel stained with EZ-vision® Bluelight DNA dye. Amplification of 16S rRNA gene and sequencing were done at Inqaba Biotech, South Africa.

Genomic sequence data analysis
Sequences were manually edited by using BioEdit (v.7.2.5) and were then compared with 16S rRNA sequences at National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). All sequences, including 5 retrieved from NCBI database were aligned by using Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm. Phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA 7) software with neighbor-joining method (Touijeret al. 2019).

Results and Discussion

Isolation and primary screening of cellulolytic bacteria
A total of 41 pure colonies of bacteria were isolated from the samples collected on NA plates. Among them, 17 isolates were found to hydrolyze CMC, while the remaining 24 isolates could not hydrolyze CMC. Halo zones due to CMC hydrolysis for representative isolates are shown in Figure 1. Isolate designated as isolate 8 obtained from a point before treatment pond showed highest cellulolytic index of 4.93 ± 0.10 and the lowest cellulolytic index value of 0.54 ± 0.06 was observed from isolate 2 obtained from preliminary treatment pond (Figure 2). Other isolates which could hydrolyze CMC may have missed out possibly due to culturing conditions used in the laboratory, which were not exactly the same as the environmental conditions from which samples were collected. The results obtained from this study support previous studies that plate assay using Gram's iodine is an easy and rapid method for screening of large numbers of cellulase-producing microorganisms (Kasana et al. 2008, Naresh et al. 2019).



E.coli *B. subtilis* Isolate 1 Isolate 12

Figure 1: Photographs of CMC agar plates depicting halo zone of isolate 1 and 12, negative control (*E. coli*) and positive control (*B. subtilis*) after 72 hours of incubation.

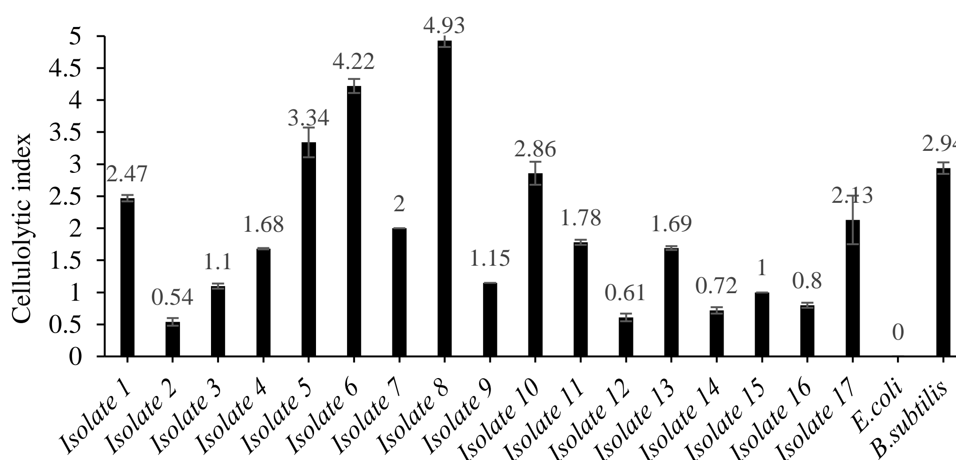


Figure 2: Cellulolytic indices of 17 obtained cellulase-producing bacteria. Data on cellulolytic index are shown as mean ± standard deviation.

Enzyme assays

The highest endoglucanase (0.0701 ± 0.010 U/ml), exoglucanase (0.0081 ± 0.001 U/mL) and β-glucosidase (0.0738 ± 0.002 U/mL) activities from submerged fermentation culture were observed from isolate 8, isolate 1 and isolate 12, respectively as shown in Figure 3. Only five isolates (1, 11, 12, 14 and 15) were found to express all the three cellulase components suggesting that these isolates could be of industrial potential for cellulose hydrolysis. The observed cellulases (endoglucanase, exoglucanase and β-glucosidase) activities from these five isolates, were however, lower than the previous reported endoglucanase (2.02 U/ml), exoglucanase (2.11 U/mL) and β-glucosidase (2.08 U/mL) activities from *Bacillus thuringiensis* under optimized conditions (Shilpa and Pethe 2017). Therefore, it can be argued that cellulases production can be increased through optimization of various factors such pH value, growth time, temperature, and introduction of inducers in fermentation medium (Sethi et al. 2013).

The maximum endoglucanase activity (0.0701 ± 0.010 U/mL) observed from isolate 8 is in agreement with other unoptimized endoglucanase activity of 0.07 U/mL from *Bacillus subtilis* AS3 reported by Deka et al. (2011). Interestingly, isolate 12 which had low cellulolytic index value (0.61 ± 0.06) was found with higher endoglucanase activity (0.0512 ± 0.004 U/mL) compared to some

other isolates. Lowest index value of this isolate may have been attributed to its poor capability to utilize CMC on the semi-solid media. It has been demonstrated experimentally that the rates of carbon sources assimilation have significant effects on both microorganisms' growth and enzyme activities (Singh et al. 2017).

For exoglucanase activity, only five isolates (1, 11, 12, 14 and 15) expressed this component, yet in lower amounts compared to endoglucanase and β-glucosidase. The results obtained in this study are consistent with other findings of Sinegani and Mahohi (2010), Soares Júnior et al. (2013) and Motsewabangwe (2016) who also reported lowest exoglucanase activities in comparison to other cellulase components. It can be argued that poor activity of exoglucanase component could be due to the fact that avicel was found to sink at the bottom of the test tubes as the reaction was carried out under non-shaking conditions. This may have reduced its accessibility to crude enzyme.

This study also recorded the maximum β-glucosidase activity of 0.0738 ± 0.002 U/mL from isolate 12 which was found to be higher than the previous reported activity of 0.067 U/mL by Menezes et al. (2019). Figure 3 also reveals that most isolates released β-glucosidase in higher amounts compared to endo- and exo-glucanase releases. This trend has been also observed by Jannah et al. (2018) from six bacteria species isolated from decomposed rice bran.

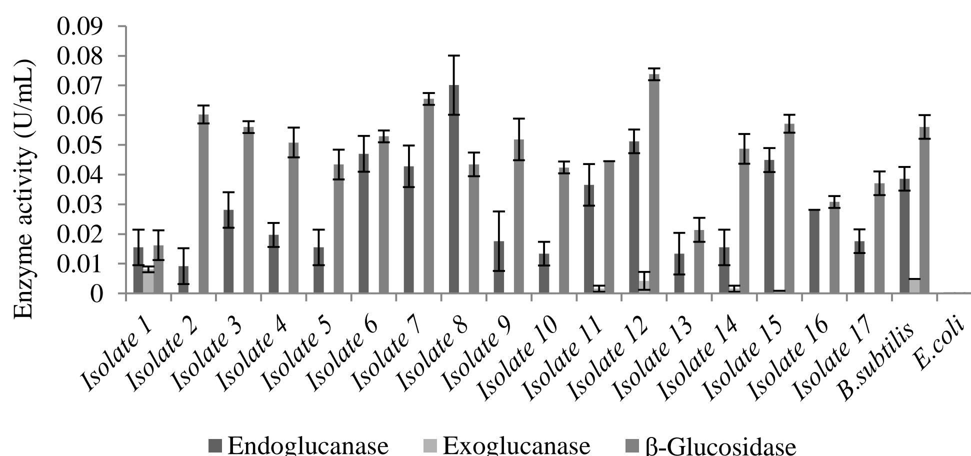


Figure 3: Endoglucanase, exoglucanase and β-glucosidase activities of 17 cellulase-producing bacteria.

Molecular characterization

Figure 4 shows the results of 16S rRNA PCR amplicons for isolates 1, 11, 12, 14 and 15 on agarose gel electrophoresis. From Basic Local Alignment Search Tool using nucleotide query (BLAST-N) (Table 1) and phylogenetic analysis (Figure 5), isolate 1 was identified as *Pararheinheimera mesophila,* grouped with other *Pararheinheimera* species in the phylogenetic tree. The other two isolates; isolates 11 and 14 were identified as *Bacillus megaterium* and they grouped with other *B. megaterium* strains. Isolates 12 and 15 were identified as *Bacillus cereus* and *Bacillus flexus* strains, respectively. They too are grouped with similar respective strains.

Although several strains of *Rheinheimera* species have been reported without cellulases activities, few strains have been documented to possess either endoglucanase or β-glucosidase activity (Baek and Jeon 2015, Sheu et al. 2018, Panda et al. 2020). So far it is only *R.*

mesophila strain IITR-13 that has been reported to exhibit both endoglucanase and β-glucosidase activities (Sheu et al. 2018). Isolate 1 obtained in this study therefore further showed the ability of *R. mesophila* to hydrolyze avicel in addition to CMC and cellobiose.

On the other hand, members of the genus *Bacillus* are well known for expression of cellulases, and several strains of *Bacillus megaterium* have been reported with cellulase activities (Al-Gheethi 2015, Sankaralingam et al. 2018). Beukes and Pletschke (2006) observed a *B. megaterium* strains with higher exoglucanase than endoglucanase activities, an observation that is contrary to isolates 11 and 14 in this study, which exhibited higher endoglucanase than exoglucanase activities. Previous findings of Chantarasiri (2015) reported *Bacillus cereus* JD0404 producing highest endoglucanase activity followed by exoglucanase and least of β-glucosidase activity. However, in contrast, isolate 12 in this study showed highest β-glucosidase activity followed by endoglucanase and least of exoglucanase activity.

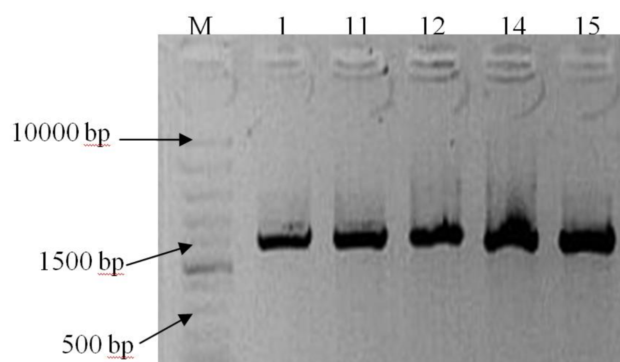


Figure 4: Agarose gel analysis of 16S rRNA PCR products for isolates 1, 11, 12, 14 and 15. Lane M is Fast DNA Ladder (New England Biolabs). The bands at 1500 bp show the sizes of the amplified PCR products.

Table 1: Isolate number, query sequence length, GenBank accession number, E-value, sequence similarity and closest species

Isolate	Query sequence length (bp)	GenBank accession number	E-Value	Sequence similarity	Closest species
Isolate 1	1390	MT833292	0.0	99.71%	*Pararheinheimera mesophila* strain IITR-13
Isolate 11	1415	MT833293	0.0	100.00%	*Bacillus megaterium* NBRC 15308 = ATCC 14581
Isolate 12	1413	MT833294	0.0	95.41%	*Bacillus cereus* strain IAM 12605
Isolate 14	1418	MT833295	0.0	100.00%	*Bacillus megaterium* NBRC 15308 = ATCC 14581
Isolate 15	1423	MT833296	0.0	99.65%	*Bacillus flexus* strainIFO15715

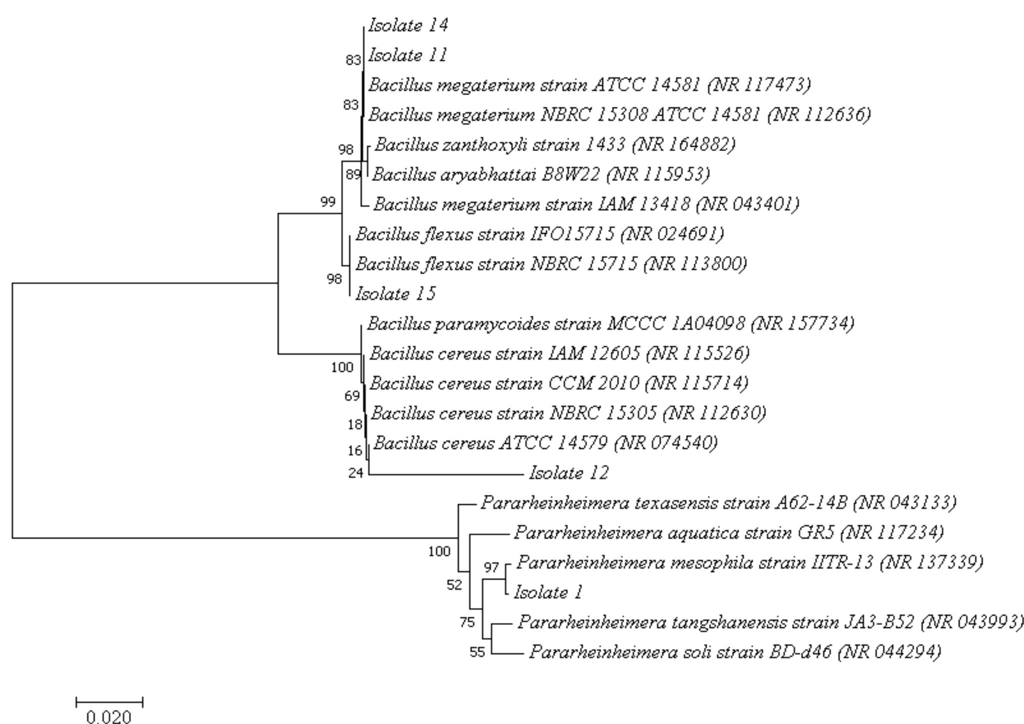


Figure 5: Phylogenetic analysis of 16S rRNA gene sequences of isolates 1, 11, 12, 14 and 15 with retrieved sequences from BLAST results.

Conclusion

The present study focused on screening and molecular characterization of cellulolytic bacteria isolated from paper mill effluent. Out of 17 cellulolytic bacteria obtained, crude enzyme extracts of only five isolates expressed all three cellulase components. This suggests that the research site chosen was a potential site for obtaining bacteria strains with expected enzymes expressions. On 16S rRNA gene sequences analyses isolates 1, 12 and 15 were identified as *Pararheinheimera mesophila*, *Bacillus cereus* and *B. flexus,* respectively, while isolates 11 and 14 were identified as *Bacillus megaterium.* The findings pave way for exploitation of native microbial cellulase enzymes for industrial applications such as biofuel production and waste management. Further research is needed to first scale up cellulase production through optimization of culture conditions and purification of the enzymes before examining its full potential for industrial applications.

Acknowledgements

We acknowledge Mr. Ferdinandi Patrick of Department of Molecular Biology and Biotechnology, University of Dar es Salaam for his assistance in laboratory enzyme assays.

Conflict of Interest: None declared.

References

Akhtar NA, Goyal D and Goyal A 2016 Biodiversity of cellulase producig bacteria and their applications. *Cell. Chem. Technol.* 50(9): 983-995.

Al-Gheethi AAS 2015 Recycling of sewage sludge as production medium for cellulase by a *Bacillus megaterium* strain. *Int. J. Recycl. Org. Waste. Agric.* 4(2): 105-119.

Arimurti S, Nurani Y, Ardyati T and Suharjono S 2017 Screening and identification of indigenous cellulolytic bacteria from Indonesian coffee pulp and investigation of its caffeine tolerance ability. *Malays. J. Microbiol.* 13(2): 109-116.

Baek K and Jeon CO 2015 *Rheinheimera aestuari* sp. nov., a marine bacterium isolated from coastal sediment. *Int. J. Syst. Evol. Microbiol.* 65(8): 2640-2645.

Beukes N and Pletschke BI 2006 Effect of sulfur-containing compounds on Bacillus cellulosome-associated 'CMCase' and 'Avicelase' activities. *FEMS Microbiol. Lett.* 264(2): 226-231.

Chantarasiri A 2015 Aquatic *Bacillus cereus* JD0404 isolated from the muddy sediments of mangrove swamps in Thailand and characterization of its cellulolytic activity. *Egypt. J. Aquat. Res.* 41(3): 257-264.

Deka D, Bhargavi P, Sharma A, Goyal D, Jawed M and Goyal A 2011 Enhancement of cellulase activity from a new strain of *Bacillus subtilis* by medium optimization and analysis with various cellulosic substrates. *Enzyme Res.* 2011: 151656.

Ferbiyanto A, Rusmana I and Raffiudin R 2015 Characterization and identification of cellulolytic bacteria from gut of worker *Macrotermes gilvus. HAYATI J. Biosci.* 22(4): 197-200.

Garate NB 2017 *Ancestral sequence reconstruction for protein engineering: improving celulases for biomass hydrolysis.* PhD dissertation, University of the Basque Country.

Gusakov AV 2011 Alternatives to *Trichoderma reesei* in biofuel production. *Trends. Biotechnol*. 29(9): 419-425.

Jannah A, Aulanniam A, ArdyatiT and Suharjono S 2018 Isolation, cellulase activity test and molecular identification of selected cellulolytic bacteria indigenous rice bran. *Indones. J. Chem.* 18: 514-521.

Kasana RC, Salwan R, Dhar H, Dutt S and Gulati A 2008 A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Curr. Microbiol.* 57(5): 503-507.

Kiio IK, Jackim MF, Munyali WB and Muge EK 2016 Isolation and characterization of a thermostable cellulase from *Bacillus licheniformis* strain vic isolated from geothermal wells in the Kenyan Rift Valley. *Open. Biotechnol. J.* 10(1): 198-207.

Li YH, Zhang XY, Zhang F, Peng LC, Zhang DB, Kondo A and Zhao XQ 2018 Optimization of cellulolytic enzyme components through engineering *Trichoderma reesei* and on-site fermentation using the soluble inducer for cellulosic ethanol production from corn stover. *Biotechnol. Biofuels* 11: 1-14.

Mahjabeen F, Khan S, Choudhury N, Hossain MM and Khan TT 2016 Isolation of cellulolytic bacteria from soil, identification by 16S rRNA gene sequencing and characterization of cellulase. *Bangladesh. J. Microbiol*. 33(1-2): 17-22.

Menezes FSR, Cruz GGD, Lopes MDO, Nelson DL, Martins TQG and Laia MLD 2019 Evaluation of endoglucanase and β-glucosidase production by bacteria and yeasts isolated from a eucalyptus plantation in the cerrado of Minas Gerais. *Rev. Ambient. Água.* 14(4): 1-9.

Moe ST, Janga KK, Hertzberg T, Hägg MB, Øyaas K and Dyrset N 2012 Saccharification of lignocellulosic biomass for biofuel and biorefinery applications–a renaissance for the concentrated acid hydrolysis?. *Energy. Procedia* 20: 50-58.

Motsewabangwe RK 2016 *Bioprospecting for cellulase enzymes of Soil-Borne Bacteria for possible application into Bioethanol production.* PhD dissertation, North-West University, South Africa.

Naresh S, Kunasundari B, Gunny AAN, Teoh YP, Shuit SH, Ng QH and Hoo PY 2019 Isolation and partial characterisation of thermophilic cellulolytic bacteria from north Malaysian tropical mangrove soil. *Trop. Life Sci. Res*. 30(1): 123-147.

Ochieng OR 2014 *Characterization of extracellular cellulolytic and xylolytic*

enzymes from organic waste degrading bacteria. PhD thesis, Kenyatta University, Kenya.

Panda AN, Ray L, Mishra SR and Raina V 2020 *Rheinheimera pleomorphica* sp. nov., a novel alkali-tolerant bacteria isolated from Chilika Lake, India. *Curr. Microbiol.* 77(1): 158-165.

Qian Y, Zhong L, Gao J, Sun N, Wang Y, Sun G and Zhong Y 2017 Production of highly efficient cellulase mixtures by genetically exploiting the potentials of *Trichoderma reesei* endogenous cellulases for hydrolysis of corncob residues. *Microbial Cell Factories* 16(1): 207.

Sankaralingam S, Palpperumal S, Harinathan B, Kathiresan D, Shankar T and Sundarapandian S 2018 Screening and characterization of cellulase by *Bacillus megaterium* isolated from marine sediments and its antimicrobial activity. *Bol. Latinoam. Caribe Plant. Med. Aromat.* 17(5): 646-653.

Sethi S, Datta A, Gupta BL and Gupta S 2013 Optimization of cellulase production from bacteria isolated from soil. *Int. Sch. Res. Notices* 2013: 1-7.

Sheu SY, Chen WT, Young CC and Chen WM 2018 *Rheinheimera coerulea* sp. nov., isolated from a freshwater creek, and emended description of genus *Rheinheimera Brettar* et al. 2002. *Int. J. Syst. Evol. Microbiol.* 68(7): 2340-2347.

Shilpa L and Pethe AS 2017 Isolation and screening of cellulolytic bacteria from soil and optimization of cellulose production. *Int. J. Life Sci*. 5(2): 277-282.

Sinegani AAS and Mahohi A 2010 Soil water potential effects on the cellulase activities of soil treated with sewage sludge. *Plant.Soil. Environ.* 56(7): 333-339.

Singh V, Haque S, Niwas R, Srivastava A, Pasupuleti M and Tripathi CKM 2017 Strategies for fermentation medium optimization: an in-depth review. *Front. Microbiol.* 2087(7): 1-10.

Singhania RR, Patel AK, Sukumaran RK, Larroche C and Pandey A 2013 Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. *Bioresour. Technol.* 127: 500-507.

Smith AD, Landoll M, Falls M and Holtzapple MT 2010 Chemical production from lignocellulosic biomass: thermochemical, sugar and carboxylate platforms. In *Bioalcohol Production*. Woodhead Publishing, 391-414.

Soares Júnior FL, Dias ACF, Fasanella CC, Taketani RG, Lima AODS, Melo IS and Andreote FD 2013 Endo-and exoglucanase activities in bacteria from mangrove sediment. *Braz. J. Microbiol.* 44(3): 969-976.

Sukumaran RK, Singhania RR and Pandey A 2005 Microbial cellulases-production, applications and challenges. *J. Sci. Ind. Res.* 64: 832-844.

Taha M, Foda M, Shahsavari E, Aburto-Medina A, Adetutu E and Ball A 2016 Commercial feasibility of lignocellulose biodegradation: possibilities and challenges. *Curr. Opin. Biotechnol.* 38: 190-197.

Touijer H, Benchemsi N, Ettayebi M, Idrissi AJ, Chaouni B and Bekkari H 2019 Thermostable cellulases from the yeast *Trichosporon* sp. *Enzyme Res*. 2019: 1-6.

Yang B, Dai Z, Ding SY and Wyman CE 2011 Enzymatic hydrolysis of cellulosic biomass. *Biofuels* 2(4): 421-449.

Zabed H, Sahu JN, Boyce AN and Faruq G 2016 Fuel ethanol production from lignocellulosic biomass: an overview on feedstocks and technological approaches. *Renew. Sust. Ener. Rev.* 66: 751-774.

Zhang YHP, Hong J and Ye X 2009 Cellulase assays. In: Mielenz J (Eds) Biofuels. Methods in Molecular Biology (Methods and Protocols) 581: 213-231.