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Isolation of Cellulose-Degrading Endophyte from *Capsicum chinense* and Determination of its Cellulolytic Potential

Angkita Sharma [1], Pooja Singh [1], Bidyut Kumar Sarmah [2], Shoma Paul Nandi [1,*]

[1] Amity Institute of Biotechnology, Amity University, Noida-201313, Uttar Pradesh
[2] Assam Agricultural University (AAU) Jorhat-785013, Assam
* Correspondence: spaul@amity.edu;

Scopus Author ID 36731454500
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Abstract: Knowledge in the field of bacterial endophytes associated with the plant *Capsicum chinense* is negligible. So in order to characterize the endophytic population in the targeted plant, different accessions of *C. chinense* plant were procured from different agro-climatic zones of India. Bacterial endophytes were isolated by using standard protocols. After isolation of the endophytes, a biochemical identification study was performed using the standard key. Secondary metabolites of these bacterial species were studied for their economic importance. One isolate of cellulose-degrading bacteria (CDB) was isolated from the roots of *C. chinense*. The cellulase activity of the endophyte, containing cellulose Congo Red agar. Finally, enzyme assays for the cellulase (endoglucanase) and filter paper cellulase or FPC assay was studied. The maximum clearing zone for the isolate was 50mm, and the hydrolytic capacity (HC) was found to be 5.96. The endoglucanase activity of 0.95 IU/mL and the filter paper Filter Paper Cellulase (FPCase) activity was found to be 0.25 IU/mL. The importance of the study is attributed to the fact that this is the first-ever study of the enzymatic activity of endophytic bacteria isolated from *C. chinense* collected from North-East India.

Keywords: *Capsicum chinense;* bacterial endophytes; secondary metabolites; cellulolytic activity; cellulase enzyme.

1. Introduction

Plants live closely associated with the microorganisms that are present in their vicinity, and more specifically, so with the ones that live internally. Endophytes are microbes, both fungi, and bacteria, which reside in an asymptomatic manner in the roots and aerial tissues of plants [1]. Various fungal and bacterial endophytes network with higher plant phylum. Interactions with mutualistic interests that led to malleable profits for the two species sometimes evolved to a higher level of intricate forms that involved more than one partner [2, 3]. The north-eastern region of India, which is a major biodiversity hot spot areas of the world, shelters a gamut of plants and their associated endophytes [4]. Endophytic bacteria increase the host plant's resistance to biotic and abiotic stresses by producing diverse bioactive compounds, such as diterpenes, flavonoids, alkaloids, and isoflavonoids. Some endophytic bacteria endorse the increase of secondary metabolite production of the host plants, which include important medicinal components or environmentally friendly compounds.

Plant biomass is considered the most profusely available organic carbon source present in the biosphere. A greater part of this carbon remains confined in cell wall polymers. The very complex structure of the polymer is attributed to the presence of cellulose, hemicelluloses, and lignin, along with pectins and structural proteins. Thus, the industrial breakdown of this material to simple forms require extreme physicochemical environments and/or a lot of diverse enzymes [5].

The enzyme system of cellulose manages cellulolysis, which is a naturally occurring biological process. There are three different classes of soluble extracellular enzymes in the cellulase enzyme system, namely: 1, 4-β-exoglucanase, 1, 4-β-endoglucanase, and β-glucosidase (Cellobiase). The enzyme endoglucanase is accountable for the arbitrary snipping of the glycosidic bonds (β-1, 4). The enzyme exoglucanase, on the other hand, is needed for breach of the non-reducing side of the cellulose chain. Eventually, it snips off the central fibrils off the crystalline form of cellulose, when β-1, 4-glucosidase breaks cellobiose along with cellodextrin forming glucose [6, 7]. Interaction of these enzymes only makes it possible to completely hydrolyze cellulose to glucose [8, 9] or a complete degradation to H_2O and CO_2. Cellulase enzyme, because of its huge potential, has been employed in many industrial processes, such as plant waste management and biofuels [10, 11]; triphasic biomethanation [12] bioethanol [13, 14], etc.

Bhut jolokia, scientific name *Capsicum Chinense* Jaqc, from the family *Solanaceae* is placed among the hottest chilies along with *'Trinidad Moruga Scorpion'* and is reported to be an indigenous cultivar, which mostly grows in Nagaland, Manipur and the Brahmaputra flood plain of Assam. *C. chinense* is a well-known medicinal plant, which is used in traditional medication as an excellent source for Vitamin C (Ascorbic acid), carotenoid, Vitamin E (tocopherols), capsaicinoids, and flavonoids. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) along with dihydrocapsaicin are considered the key capsaicinoids found in hot peppers. Homodihydrocapsaicin, nordihydrocapsaicin, and homocapsaicin are the ones present in lower amounts [15]. A measurement to calculate the pungency was established by Wilbur Scoville; total capsaicinoids are able to be altered to Scoville heat units [16]. *C. chinense* is attributed to having a lot of medicinal properties with immense pharmacological actions. These include antitumor, antibacterial, anti-arthritic, anti-inflammatory, and antioxidant properties. However, the environmental aspects of this plant and its associated endophytes have not been studied so far.

The current work focusses on isolation and identification of cellulose-degrading bacterial endophytes from the roots of *Capsicum chinense* plant collected from North-east India.

2. Materials and Methods

2.1. Collection site.

C. chinense plant was collected from the remote areas of Arunachal Pradesh, in the summer season, in the month of April. Samples were aseptically transferred into different zipped sterile polythene bags and then transported to the laboratory at room temperature [17].

2.2. Isolation of endophyte.

Endophyte was isolated using a standard protocol. Surface sterilization was done using 70% ethanol and 4% sodium hypochlorite solution. To remove any kind of adherents on the

plant surface, it was washed properly under running tap water. After drying, the plant roots were cut into small segments approximately 1-2 cm in length. This whole procedure was carried out in laminar airflow. Plant segments were rinsed with 75% ethanol for 1 min and then with 4% sodium hypochlorite solution for 10 min. This was followed by immersing the segments in 75% ethanol for 1 min. Finally, all the plant segments were rinsed thrice with sterilized distilled water and were allowed to air dry. The sample was then ground using mortar and pestle, and the extract was plated in Nutrient Agar (NA) plates. The plates were incubated at 37ºC for bacteria overnight [18].

2.3. Morphological and Biochemical identification of the isolated bacteria.

In order to confirm the cell morphology and the classification of the bacterial endophyte, general morphology was studied, and Gram staining was done with 100X optical microscopic visualization. Conventional biochemical tests, including the Indole test, Methyl red, Voges- Proskauer, Citrate test, Triple-Sugar Iron test, were done for the identification of the isolate [19].

2.4. Total protein estimation.

The total protein estimation of the isolate was done by Lowry method of protein estimation. Reagents required are BSA stock solution (1mg/ml), the analytical reagents include (a) 50ml of 2% sodium carbonate with 50ml of 0.1N NaOH, (b) 10ml of Copper sulphate (1.56%) solution with 10ml of sodium-potassium tartrate (2.37%) solution. This analytical reagent was prepared by mixing 2ml of the solution (b) with 100ml of solution (a). The Folin-Ciocalteau reagent (1N) was obtained by diluting commercial reagent (2N) with an equal volume of water freshly prepared every time [20, 21]. The total protein concentration in the sample was recorded by reading the absorbance at 750nm of the end product of the Folin reaction against a standard curve of Bovine Serum Albumin (BSA). 0.05g of BSA was weighed and added to a 500mL volumetric flask with ddl water. The volume was adjusted to 500mL with ddl water. The final concentration of the BSA stock was made to 100mg /L.

2.5. Screening of Cellulose-degrading capacity of the isolated bacteria.

Bacterial endophyte isolates feeding on cellulose as a carbon source was isolated on agar media enriched with cellulose with a composition of KH_2PO_4 0.50 g along with $MgSO_4$ 0.27 g. Other components include gelatin 2.0 g; cellulose 2.0 g; and agar 15 g. 1Ldistilled water was added, and the pH was maintained at 6.8–7.2. For the determination of the isolate's capacity to degrade cellulose, cellulose CongoRed agar media was used, which was enriched with KH_2PO_4 0.5.0 g and $MgSO_4$ 0.25 g. 15 g of agar was then added to 0.2g Congo-Red, along with 2g gelatin and 2g cellulose. 1L distilled water was added with a pH maintained at 6.8–7.2. Congo-Red, which indicates the cellulose-degrading ability of the isolate in an agar media, is a subtle and quick screening test for the cellulose-degrading capability of the endophyte. The quantitative estimation of the degradation ability of the isolate was done by computing the hydrolysis capacity [22, 23].

2.6. Enzyme Production and estimation.

The isolated cellulose-degrading bacteria (CDB) was cultured at 37ºC at 150 rpm for enzyme production with a media composition of KH_2PO_4 0.5.0 g and $MgSO_4$ 0.25 g, along

with gelatin 2.0 g; 1 liter distilled water was added containing Whatman filter paper 1 with a measurement of 1 × 6 cm strip or 0.050 g in 20 mL (pH of 6.8–7.2). The optical density (OD_{600nm}) was measured every 3 hours for 72 hours. The cellulase was harvested at the optimum incubation time with the value of the highest cellulase activity. The broth culture incubated for three days period was then centrifuged at around 5000 rpm for 10 minutes at 4ºC. The crude enzyme was the supernatant that was collected, which was then kept at 4ºC for further assay studies [24, 25, 26].

2.7. Enzyme Assay.

The cellulose-degrading activity of the isolate was checked by computing, reducing sugar concentration formed after the degradation of the filter paper. The enzyme assay was carried out according to the approvals of the International Union of Pure and Applied Chemistry (IUPAC) commission [31] on biotechnology. The endoglucanase activity was calculated by incubation of 0.5 mL of the supernatant obtained with 0.5 mL amorphous cellulose (2%) in a buffer system (sodium citrate buffer with a pH of 4.8) at a temperature of 50 degrees for 30 min. Similarly, the FPC enzymatic activity was calculated by the incubation of the supernatant (0.5 mL) with 1.0 mL of the buffer (sodium citrate buffer) having 0.05 M at pH4.8. This was enhanced with the Whatman no.1 filter paper cut in the measurements 1.0 × 6.0 cm. After the incubation process for 1 h at a temperature of 50ºC, 3, 5-dinitrosalicylic acid (DNS) reagent (3 mL) was added to the reaction to end it along with the reaction mixture (1 mL). The reducing sugars were assessed with the help of a spectrophotometer along with DNS reagent [27] with glucose used as standards. The enzymatic activity was defined in international units (IU). The amount of enzyme releasing 1 µmol reducing sugars (measured as glucose) per mL per minute measures one unit of enzymatic activity [23, 28, 29, 30, 31].

2.8. Statistical analysis.

A one-way ANOVA was carried out to check the significance of the results at P (<0.005) between the two groups, and the tests were run in triplicates.

3. Results and Discussion

3.1. Isolation, screening, and identification of the cellulase producing endophyte.

Out of the total population of endophytes isolated from the roots of *C. chinense*, five showed cellulolytic potential, of which one showed the most potential. The study was carried forward with this particular isolate, termed Cb1 (Fig 1).

A B

Figure 1. A. Different bacterial endophytes from *C chinense*; B. Isolate Cb1.

The morphological attributes of the isolated strain Cb1 are given in Table 1.

Table 1. Morphological characterization of the bacterial isolate Cb1.

Isolate code	Cb1
Color	Orange Yellow
Opacity	opaque
Surface	smooth
Form	circular
Elevation	raised
Margin	entire

The biochemical characteristics of the isolate are given in Table 2.

Table 2. Biochemical characterization of the bacterial isolate.

Isolate code	**Cb1**	**Images**
Gram stain	Negative	-
Shape	rod long	-
Sulphide	Negative	
Motility	Negative	-
Indole	Negative	
Methyl red	Positive	
Voges Prauskeur	Positive	
Citrate utilization	Positive	

Gelatin test	Negative	
Triple Sugar Iron (TSI) test	A/NC	

A/NC stands for Acid/No Change = Carbohydrate fermentation without growth in the anaerobic slant.

Based on the morphological and biochemical studies, the isolated strain was identified to be *Hafnia* sps.

3.2. Total protein estimation.

The BSA (100mg/L) dilutions with absorbance at 750 nm were plotted in a graph, and the total protein of the isolate at OD_{750nm} was found to be 1.850 after 24 hours of incubation and plotting from the BSA standard graph, the total protein in the isolate was found to be 410 mg/L.

3.3. Cellulase degrading assay.

The cellulolytic capacity of the isolate was tested on the Congo-Red agar medium, which showed gave a zone of clearance around the inoculum (Fig 2).

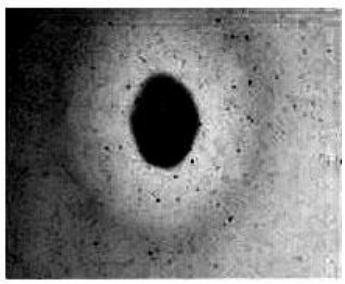

Figure 2. Congo-Red agar plate showing the zone of clearance.

The cellulolytic capacity of the isolate was put to comparison with that of a positive control *Bacillus* sps. The results are shown in Table 3, followed by the graph (Fig 3).

Table 3. The comparative study between the cellulolytic activity of Cb1 and *Bacillus* sps.

Source	**Isolate Number**	**Maximum clearing zone (mm)**	**Average HC value**	**Endoglucanse activity range (IU/mL)**	**FPCase activity range (IU/mL)**
C. chinense	Cb1	50	5.96	0.95	0.25
Bacillus sps. (Positive control)	Cb0	40	3.51	0.750	0.09

Here, the HC value = ratio of Hydrolytic Capacity; FPCase activity=Filter Paper Cellulase activity.

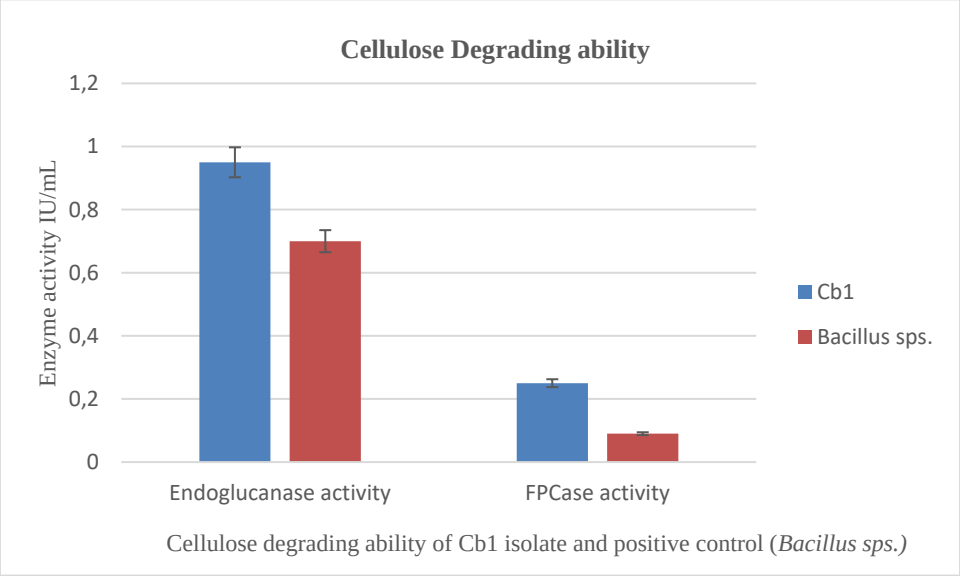

Figure 3. The comparative enzyme activity of endophyte Cb1 and *Bacillus* sps (Cb0).

3.4. Statistical study.

The one-way ANOVA results showed that the data was significant at P (<0.005) between the two groups, with the results run in triplicates.

3.5. Discussion.

The current study is the first approach to test the cellulolytic activity of bacterial endophytes isolated from *C. chinense* from North-East India. A recent study showed that bacterial species like *Staphylococcus sps* along with *Escherichia coli, Enterobacter cancerogenus, S. Bacillus subtilis* possess cellulolytic activity [32]. Another study showed such activity in *Bacillus thuringeinsis, Brevibacillus brevis, Brevibacillus parabrevis,* and *Bacillus pumilus, B. licheniformis, B. methylotrophicus. Burkholderia sp.* [33, 34, 35, 36, 37]. The endoglucanase from newly isolated cellulolytic bacteria *B. safensis* was successfully cloned and expressed in *B. subtilis* [38]. *B. methylotrophicus* extract that contains amylase is a potential candidate in environmental remediation processes for future use. [39].

Plant associated endophytes have always been a fascinating area of research for scientists. The North-eastern region of the country is known for its vast flora and its closely related endophytes. The microbial diversity of this region features a high level of endemism, and the region is considered a biodiversity hotspot. In this study, bacterial endophytes were isolated from the roots of *C. chinense* collected from the remote regions of North-east India. Out of all the isolates, one isolate showed the maximum cellulolytic activity. The cellulolytic capability of a microorganism is an area of immense potential in the field of environmental microbiology. The bacterial endophyte isolated from the roots of *C. chinense* was characterized both morphologically and biochemically and was placed under the genus *Hafnia* sp. The screening of this isolate for its cellulolytic potential by the use of the Congo-Red iodine test, followed by DNS test, showed its hydrolytic capability and cellulase enzyme production. Bacterial growth was confirmed by the absorbance at 600 nm. The maximum clearing zone for the isolate was 50mm, and the hydrolytic capacity (HC) was found to be 5.96. The endoglucanase activity ranged from 0.95 IU/mL, and the filter paper Fpase activity was found to be 0.25 IU/mL

The cellulolytic activity of the isolate was then compared with that of *Bacillus sps.* taken as a positive control. The results revealed that our isolate had a greater zone of clearance of 50mm as compared to *Bacillus*, which was 40mm. Moreover, the isolate had greater endoglucanase and FPCase activity as compared to the *Bacillus* sps.

4. Conclusions

This is the first report on the cellulolytic activity of bacterial endophytes isolated from the roots of *Capsicum chinense* collected from North-East India. The current study shows the presence of endophytes in *C. chinense* roots collected from the North-east region of India. The study also shows the cellulolytic activitiy of the endophyte, which supports the presence of commercially important enzymes. In regard to the cellulolytic potential of the bacterial endophyte harboring in the root tissues of *C. chinense*, the conservation of such an economically important plant is very crucial. Further research is required to scale up the physiological and biochemical attributes of the bacterial endophytes from *C. chinense* such that cellulolytic potential can be further enhanced.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Frey-Klett, P.; Burlinson, P.; Deveau, A.; Barret, M.; Tarkka, M.; Sarniguet, A. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol Mol Biol Rev* **2011**, *75*, 583–609, https://doi.org/10.1128/mmbr.00020-11.
2. Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* **2015**, *79*, 293-320, https://doi.org/10.1128/mmbr.00050-14.
3. Tamosiune, I.; Baniulis, D.; Stanys, V. Role of Endophytic Bacteria in Stress Tolerance of Agricultural Plants: Diversity of Microorganisms and Molecular Mechanisms. In: *Probiotics in Agroecosystem*. Kumar, V.; Kumar, M.; Sharma, S.; Prasad, R. Eds. Springer Singapore: Singapore, 2017; pp. 1-29, https://doi.org/10.1007/978-981-10-4059-7_1.
4. Joshi, S.R.; Bhattacharjee, K.; Banerjee, A.; Bareh, D.A. Microbial diversity distribution in the lower belt of eastern Himalaya. *Biodiversity in Tropical Ecosystems* **2015**, 261-288.
5. Samdhu, D.K.; Bawa, S. Improvement of cellulase activity in Trichoderma. *Appl Biochem Biotech* **1992**, *34-35*, 175–192, https://doi.org/10.1007/BF02920544.
6. Shewale, J.G. β-Glucosidase: Its role in cellulase synthesis and hydrolysis of cellulose. *International Journal of Biochemistry* **1982**, *14*, 435-443, https://doi.org/10.1016/0020-711X(82)90109-4.
7. Woodward, J.; Wiseman, A. Fungal and other β-d-glucosidases — Their properties and applications. *Enzyme and Microbial Technology* **1982**, *4*, 73-79, https://doi.org/10.1016/0141-0229(82)90084-9.
8. Ryu, D.D.Y.; Mandels, M. Cellulases: Biosynthesis and applications. *Enzyme and Microbial Technology* **1980**, *2*, 91-102, https://doi.org/10.1016/0141-0229(80)90063-0.
9. Wood, T.M.; McCrae, S.I.; Bhat, K.M. The mechanism of fungal cellulase action. Synergism between enzyme components of Penicillium pinophilum cellulase in solubilizing hydrogen bond-ordered cellulose. *Biochemical Journal* **1989**, *260*, 37-43, https://doi.org/10.1042/bj2600037.

10. Lu, W.J.; Wang, H.T.; Nie, Y.F.; Wang, Z.C.; Huang, D.Y.; Qiu, X.Y.; Chen, J.C. Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process. *J Environ Sci Health B* **2004**, *39*, 871-887.
11. Mswaka, A.Y.; Magan, N. Wood degradation, and cellulase and ligninase production, by Trametes and other wood-inhabiting basidiomycetes from indigenous forests of Zimbabwe. *Mycological Research* **1998**, *102*, 1399-1404, https://doi.org/10.1017/S0953756298006789.
12. Chakraborty, N.; Sarkar, G.M.; Lahiri, S.C. Cellulose degrading capabilities of cellulolytic bacteria isolated from the intestinal fluids of the silver cricket. *Environmentalist* **2000**, *20*, 9-11, https://doi.org/10.1023/A:1006691524607.
13. Ekperigin, M.M. Preliminary studies of cellulase production by Acinetobacter anitratus and Branhamella sp. *Afr J Biotechnol* **2007**, *6*, 28–33.
14. Vaithanomsat, P.; Chuichulcherm, S.; Apiwatanapiwat, W. Bioethanol production from enzymatically saccharified sunflower stalks using steam explosion as pretreatment. *World Acad Sci Eng Technol* **2009**, *37*, 140–143, https://doi.org/10.5281/zenodo.1054974.
15. Howard, L.R.; Wildman, R.E.C. Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum annum*). In: *Handbook of nutraceuticals and functional foods.* Ed. Wildman, REC. Boca Raton, FL: CRC Press, 2007; pp. 165-191.
16. Gogoi, B. Capsicum chinense Jacq. (Bhut Jolokia) – rich source of capsaicin with wide application and economic potential. *Annals of Plant Sciences* **2017**, *6*, 1664-1667, https://doi.org/10.21746/aps.2017.8.3.
17. Ratnaweera, P.B.; de Silva, E.D.; Williams, D.E.; Andersen, R.J. Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic Fusarium sp. *BMC Complem Altern M* **2015**, *15*, https://doi.org/10.1186/s12906-015-0722-4.
18. Deeb, B.E.; Bazaid, S.; Gherbawy, Y.; Elhariry, H. Characterisation of endophytic bacteria associated with rose plant *(Rosa damascene trigintipeta*) during flowering stage and their plant growth promoting traits. *J Plant Interact* **2012**, *7*, 248-253, https://doi.org/10.1080/17429145.2011.637161.
19. Gerhardt, P.; Murray, R.G.E.; Wood, W.A.; Krieg, N.R. Methods for general and molecular Bacteriology. ASM Washington, **1994**; pp, 518.
20. Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin Phenol Reagent. *J Biol Chem* **1951**, *193*, 265-275.
21. Maitra, S.S. Experimental determination of Kinetic parameters for anaerobic digestion of MsW leachate for prediction of contaminant transport through vadose zone. In: *National convention on Biotechnology and the Environment held at NIT Durgapur*. At NIT Durgapur 2010**.**
22. Hendricks, C.W.; Doyle, J.D.; Hugley, B. A new solid medium for enumerating cellulose-utilizing bacteria in soil. *Applied and environmental microbiology* **1995**, *61*, 2016-2019.
23. Liang, Y.L.; Zhang, Z.; Wu, M.; Wu, Y. Feng. J.X. Isolation, Screening, and identification of cellulolytic bacteria from Natural Reserves in the subtropical region of China and optimization of cellulose production by *Paenibacillus terrare* ME27-1. *Biomed Res Int* **2014**, *2014*, https://doi.org/10.1155/2014/512497.
24. Saha, S.; Roy, R.; Sen, S.K.; Ray, A.K. Characterisation of cellulase-producing bacteria from the digestive tract of Tilapia, Oreochromis mossambica (Peters) and grass carp, *Ctenopharyngodon idella* (Valenciennes). *Aquac Res* **2006**, *37*, 380-8, https://doi.org/10.1111/j.1365-2109.2006.01442.x.
25. Klemm, D.; Heublein, B.; Fink, H.-P.; Bohn, A. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. *Angewandte Chemie International Edition* **2005**, *44*, 3358-3393, https://doi.org/10.1002/anie.200460587.
26. Shanmugapriya, K.; Saravana, P.S.; Krishnapriya, M.M.; Mythilli, A.; Joseph, S. Isolation, screening and partial purification of cellulose from cellulose producing bacteria. *Int J Adv Biotechnol Res* **2012**, *3*, 509-14.
27. Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* **1959**, *31*, 426–428, https://doi.org/10.1021/ac60147a030.
28. Islam, F.; Roy, N. Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. *BMC Research Notes* **2018**, *11*, https://doi.org/10.1186/s13104-018-3558-4.
29. Bhat, M.K. Celluases and related enzymes in biotechnology. *Adv Biotechnol* **2000**, *1*, 355-83. https://doi.org/10.1016/S0734-9750(00)00041-0.
30. Nandimath, A.P.; Kharat, K.R.; Gupta, S.G.; Kharat, A.S. Optimization of cellulase production for Bacillus sp. and *Pseudomonas* sp. soil isolates. *Afr J Microbiol Res* **2016**, *10*, 410-9, https://doi.org/10.5897/AJMR2016.7954.
31. Ghose, T.K. Mesurnment of cellulase activity. *Pure Appl Chem* **1987**, *59*, 257–268, https://doi.org/10.1351/pac198759020257.
32. Flimban, S.; Oh, S.-E.; Joo, J.H.; Hussein, K.A. Characterization and Identification of Cellulose-degrading Bacteria Isolated from a Microbial Fuel Cell Reactor. *Biotechnology and Bioprocess Engineering* **2019**, *24*, 622-631, https://doi.org/10.1007/s12257-019-0089-3.
33. Mohammed, R.; Salah, A.; Ahmed, B. Isolation and Identification of Cellulose Degrading Bacteria from Different Sources at Assiut Governorate (Upper Egypt). *J Ecol Heal Environ Int J* **2018**, *6*, 15-24, http://dx.doi.org/10.18576/jehe/060103.

34. Chen, Z.; Meng, T.; Li, Z.; Liu, P.; Wang, Y.; He, N.; Liang, D. Characterization of a beta-glucosidase from Bacillus licheniformis and its effect on bioflocculant degradation. *AMB Express* **2017**, *7*, https://doi.org/10.1186/s13568-017-0501-3.
35. Ma, L.; Lu, Y.; Yan, H.; Wang, X.; Yi, Y.; Shan, Y.; Liu, B.; Zhou, Y.; Lü, X. Screening of cellulolytic bacteria from rotten wood of Qinling (China) for biomass degradation and cloning of cellulases from Bacillus methylotrophicus. *BMC Biotechnology* **2020**, *20*, https://doi.org/10.1186/s12896-019-0593-8.
36. Yang, C.X.; Wang, T.; Gao, L.N.; Yin, H.J.; Lü, X. Isolation, identification and characterization of lignin-degrading bacteria from Qinling, China. *J Appl Microbiol* **2017**, *123*, 1447-1460, https://doi.org/10.1111/jam.13562.
37. Kazeem, M.O.; Shah, U.K.M.; Baharuddin, A.S.; AbdulRahman, N.A. Prospecting Agro-waste Cocktail: Supplementation for Cellulase Production by a Newly Isolated Thermophilic B. licheniformis 2D55. *Appl Biochem Biotechnol* **2017**, *182*, 1318-1340, https://doi.org/10.1007/s12010-017-2401-z.
38. Kanchanadumkerng, P.; Sakka, M.; Sakka, K.; Wiwat, C. Characterization and secretive expression in Bacillus subtilis of endoglucanase from *Bacillus safensis* isolated from freshwater swamp forest. *Walailak J Sci Technol (WJST)* **2017**, *14*, 199–213.
39. Hmidet, N.; Jemil, N.; Nasri, M. Simultaneous production of alkaline amylase and biosurfactant by Bacillus methylotrophicus DCS1: application as detergent additive. *Biodegradation* **2019**, *30*, 247-258, https://doi.org/10.1007/s10532-018-9847-8.
40. Sharma, S.; Rashmitha, CS.; Pandey, LM. Synthesis and characterization of methyl acrylamide cellulose nanowhiskers for environmental applications *Lett Appl NanoBioScience* **2020**, *9*, 880 – 884. https://doi.org/10.33263/LIANBS91.880884.
41. Bayoumy, AM.; El-Sayed ESM.; Omar A.; Ibrahim M. Emerging applications of chitosan: from biology to environment. *Biointerface Res Appl Chem* **2018**, 8, 3368 – 3380.
42. Tawfik, S.; Abd Elsalam, SH.; Nassar, SH.; Sehata, IA.; Elshemy, NS.; El-Thalouth, A. Microwave assisted chemical modification of starch in presence or absence of amylase enzyme and its application in textile printing. *Lett Appl NanoBioScience* **2019**, *8*, 762 – 769. https://doi.org/10.33263/LIANBS84.762769
43. Ezzat, HA.; Hegazy, MA.; Nada, NA.; Ibrahim, MA. Effect of nano metal oxides on the electronic properties of cellulose, chitosan and sodium alginate. *Biointerface Res Appl Chem* **2019**, *9*, 4143 – 4149. https://doi.org/10.33263/BRIAC94.143149.