Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166

ISSN 2350-8647 (Print) 2542-2901 (Online)

Research Article

Trichoderma sp. for cellulase enzyme production

Aparna Gunjal
Department of Microbiology, Dr. D. Y. Patil, Arts, Commerce and Science College, Pimpri, Pune, Maharashtra, India
(Received: 31 October 2022; Revised: 21 March 2023; Accepted: 16 May 2023)

Abstract
The enzymes have wide applications in various industries. The group of microorganisms, vizproduproduceungi, actinobacteria produces different enzymes, viz., cellulase, chitinase, amylase, protease, lecithinase, lipase, gelatinase, etc. and these enzymes have immense market demand. The work here describes the isolation of *Trichoderma* sp. and study of their colony characteristics on different agar media. The morphology images of *Trichoderma* sp. were also studied light and stereo microscope. The isolated *Trichoderma* sp. was studied for the production of cellulase. The isolated three species of *Trichoderma* were found to show production of cellulase. The cellulase production was more by *Trichoderma* sp. isolate No. 1. The cellulase production by *Trichoderma* sp. will have applications in food and other industries. The production of cellulase by *Trichoderma* sp. will be economical and eco-friendly.

***Keywords*:** Agriculture, compost, eco-friendly, economical

Introduction
Enzymes have many applications in industries. The enzymes are produced by a group of microorganisms, viz., bacteria, fungi and actinobacteria (Imran et al*.,* 2016; Li et al*.,* 2020). The fungi are known to produce amylase, cellulase, chitinase, lipase, protease enzymes. The research paper here describes isolation, characterization, and microscopic studies of *Trichoderma* sp. and to check for their ability for cellulase production. *Trichoderma* sp. has many applications in agriculture and can be used as biocontrol agent. The microbial production of enzymes will be eco-friendly and economical. Cellulase has applications in food and pharmaceutical industries. The production of cellulase enzyme by *Trichoderma* sp. is a biological approach which is eco-friendly and economical. *Trichoderma* sp. being fungi is known for good production of cellulase enzyme.

Materials and Methods
Collection of the soil samples
For the isolation of *Trichoderma*, the soil samples were collected from Manjari farm area, Pune, Maharashtra, India.

Isolation of *Trichoderma* sp.
For the isolation of *Trichoderma* sp., the soil samples (1 g) were weighed and suspended in 1 ml sterile saline. The serial dilutions were made as 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. The 0.1 mL of 10^{-3} dilution was spread plate on potato dextrose agar medium and the plates were incubated in the incubator at 30°C for eight to ten days.

Corresponding author: aparnadyp@gmail.com

Colony characteristics of *Trichoderma* sp. on different media
The spot inoculations were done of the three isolates No. 1, 2 and 3 and the colony characteristics were studied on different media viz., potato dextrose agar (PDA) [(g/L): potato: 200.0, dextrose: 20.0, agar: 30.0, DW: 1000 mL], Czapex dox (Cdox) [g/L): sucrose: 4.0, $NaNO_3$: 0.2, $MgSO_4.7H_2O$: 0.05, K_2HPO_4: 0.1, pH: 6.8, agar: 30.0, DW: 1000 mL], Sabouraud dextrose (SDA) [(g/l): peptone: 10.0, dextrose: 40.0, pH: 5.8, agar: 30.0, DW: 1000 mL], malt extract (MEA) [(g/L): malt extract: 20.0, pH: 6.5, agar: 30.0, DW: 1000 mL] and oat meal (OMA) [(g/L): oat meal: 60.0, pH:7.2, agar: 30.0, DW: 1000 mL] agar media. For the colony characteristics, the plates were incubated at 30°C for seven days.

Microscopy studies
The morphology studies were also done using light (Model: DSX1000, India) and stereo (Model: Zsm-115, India) microscopes to observe the spores, conidia, conidiophores, fertile hair and apices, mycelium, and branching tips.

Cellulase production
The fungal isolates were inoculated on the minimal agar [g/L): NH_4Cl: 3.0, NaCl: 3.0, K_2HPO_4: 0.4, sodium citrate: 3.0, glucose: 10.0, pH: 7.0, agar: 30.0, DW: 1000 mL] medium with carbon source replaced by 1% carboxymethyl cellulose (CMC) (Sigma-Aldrich, USA). The plates were incubated at 28°C for three to four days and then flooded with congo red (1%) reagent [congo red: 1.0 g, DW: 100 mL] for 5 min at room temperature. Excess stain was discarded, and the plates were destained with 1 M NaCl solution (Teather & Wood, 1982). Cellulase activity was indicated by the development of clear zone around the colony.

Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166

Results and Discussion

Isolation of the fungi

A total of three isolates were obtained which were named as No. 1, 2, and 3. These isolates were identified to be *Trichoderma* sp. based on their colony morphology studies.

Colony morphology studies of the isolates of *Trichoderma* sp. on different media

The colony morphology of isolates No. 1, 2 and 3 on PDA, Cdox, SDA, MEA and OMA (Figs. 1, 2, 3, 4, & 5) are shown in Tables 1, 2, and 3, respectively.

Table 1 Colony morphology of isolate No. 1 on different media

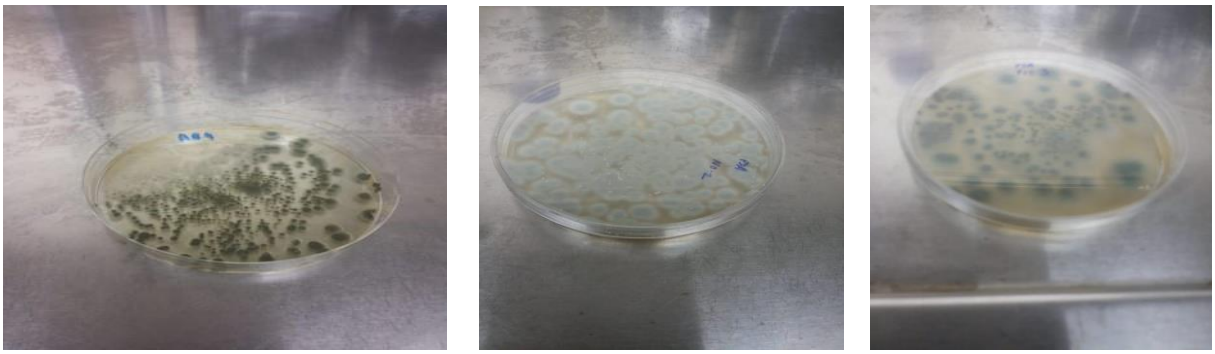
Media	Color	Margin	Texture	Hyphal thickness
PDA	White	Regular	Loose, puffy	Very thick
Cdox	White	Regular	Loose	Thin
SDA	White	Regular	Loose	Thick
MEA	White	Regular	Moderately compact	Thick
OMA	White	Regular	Loose	Thin

Table 2 Colony morphology of isolate No. 2 on different media

Media	Color	Margin	Texture	Hyphal thickness
PDA	White	Regular	Moderately compact	Thin
Cdox	White	Regular	Loose	Very thick
SDA	White	Regular	Very compact	Thick
MEA	White	Regular	Very compact	Thick
OMA	White	Regular	Very compact	Thick

Table 3 Colony morphology of isolate No. 3 on different media

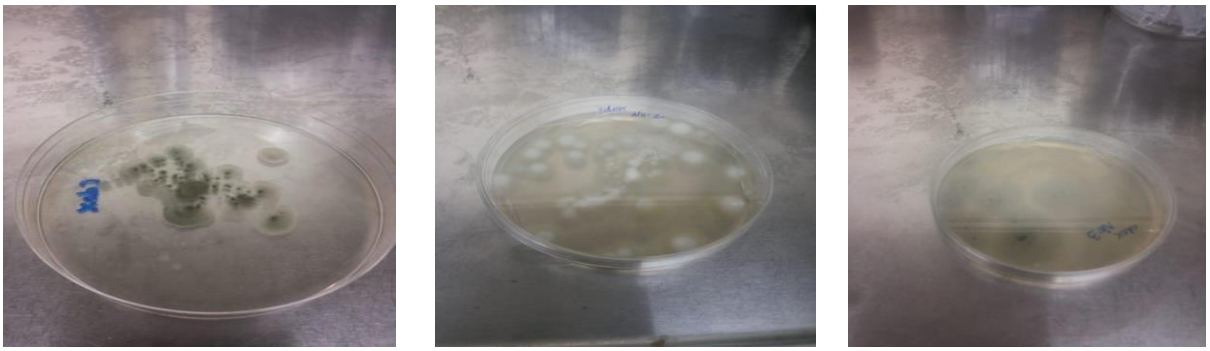
Media	Color	Margin	Texture	Hyphal thickness
PDA	White	Regular	Loose	Thin
Cdox	White	Regular	Loose	Thin
SDA	White	Regular	Loose	Thin
MEA	White	Regular	Compact	Thin
OMA	White	Regular	Very compact	Thin



(a) (b) (c)

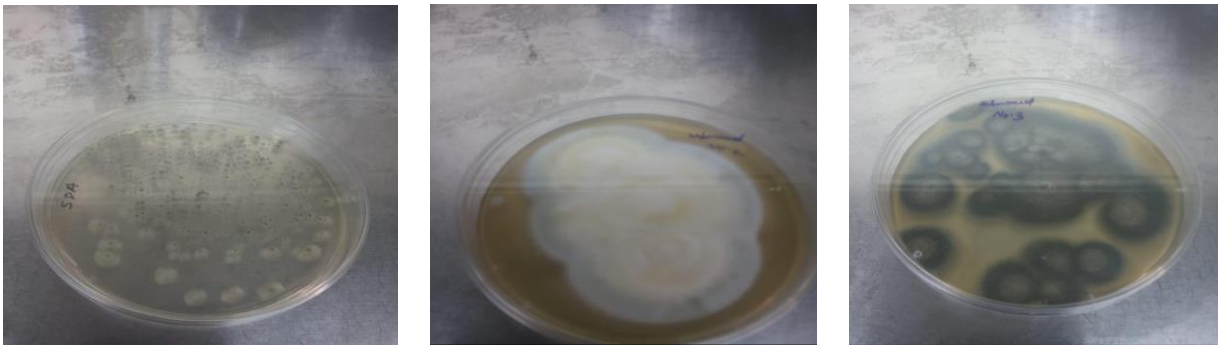
Figure 1 The figure plates a, b, and c represent growth of isolates No. 1, 2, and 3, respectively on PDA media

Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166



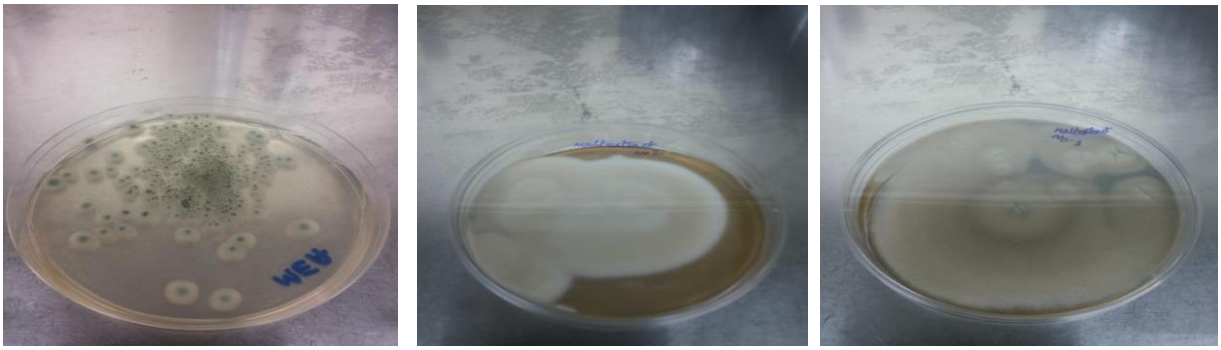
(a) (b) (c)

Figure 2 Figure plates a, b, and c represent growth of isolates No. 1, 2, and 3, respectively on Cdox agar media



(a) (b) (c)

Figure 3 Figure plates a, b, and c represent growth of isolates No. 1, 2, and 3, respectively on SDA media



(a) (b) (c)

Figure 4 Figure plates a, b, and c represent growth of isolates No. 1, 2, and 3, respectively on MEA media

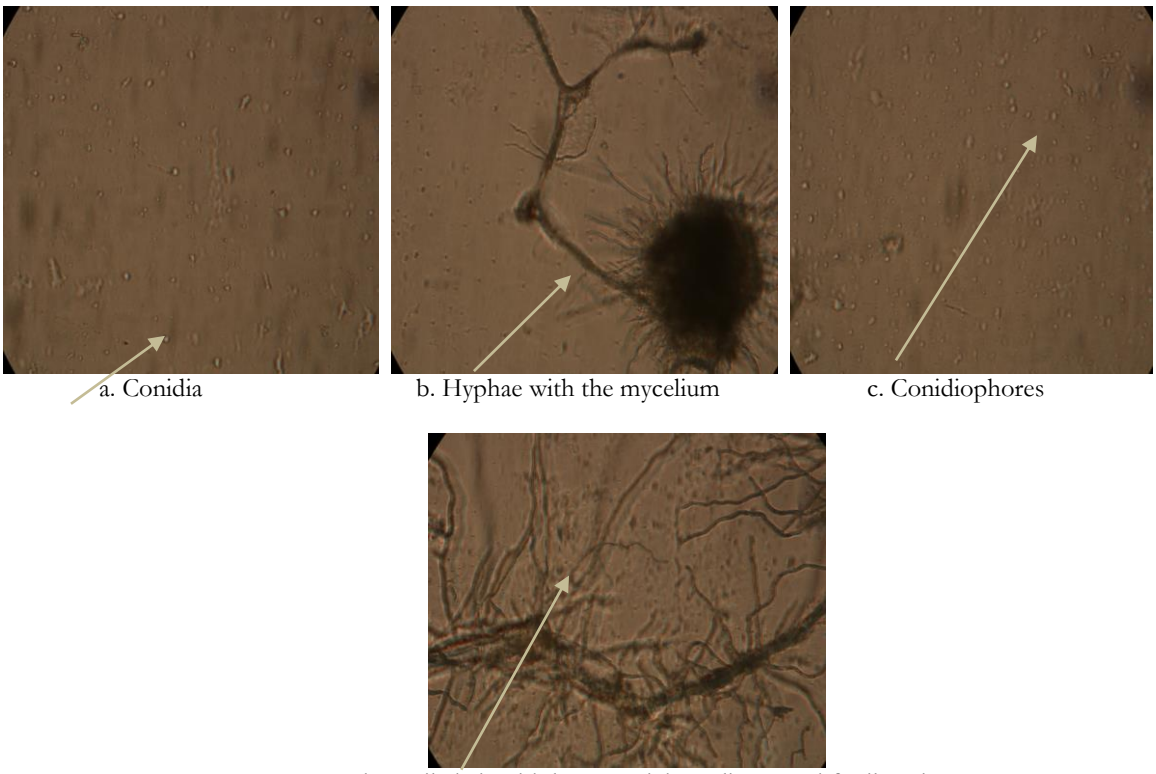
(a) (b) (c)

Figure 5 Figure plates a, b, and c represent growth of isolates No. 1, 2, and 3, respectively on OMA media.

Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166

Light and stereo microscopy studies of *Trichoderma* sp.

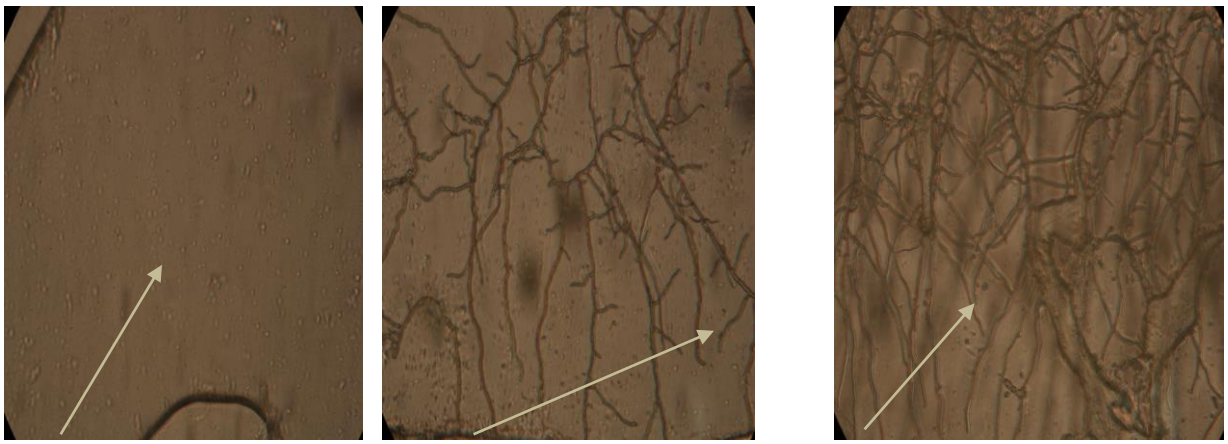
The spores, conidia, conidiophores, hyphae with mycelium, fertile hair with long fertile apices and branching tips of the three isolates of *Trichoderma* sp. (isolates No. 1, 2 and 3) were observed under light and stereo microscope. The morphology of *Trichoderma* sp. isolates No. 1, 2 and 3 using light microscope are represented in Fig. 6, 7 and 8, respectively. The morphology of *Trichoderma* sp. isolates No. 1, 2 and 3 using stereo microscope are represented in Fig. 9, 10, and 11, respectively.



a. Conidia b. Hyphae with the mycelium c. Conidiophores

d. Fertile hair with long, straight, solitary, and fertile apices

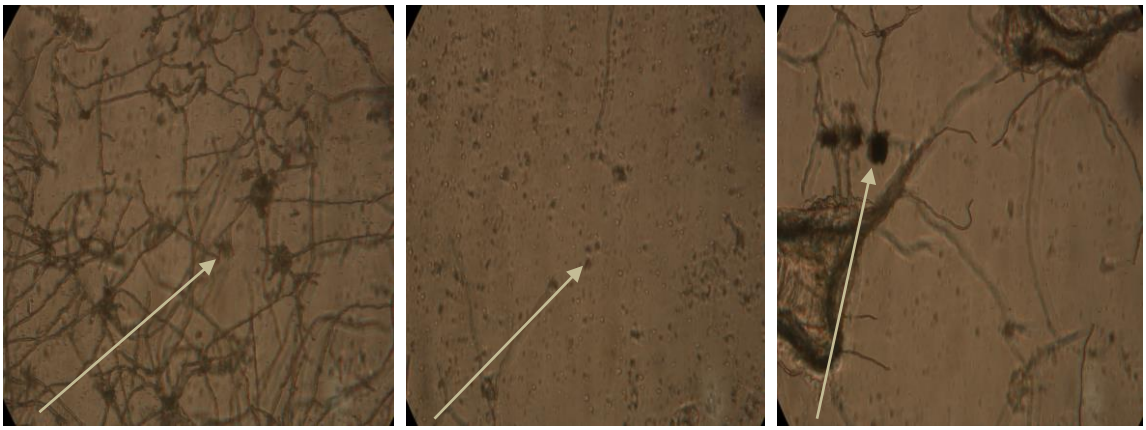
Figure 6 Figure plates a, b, c, and d represent the morphology study of isolate No. 1 using light microscope



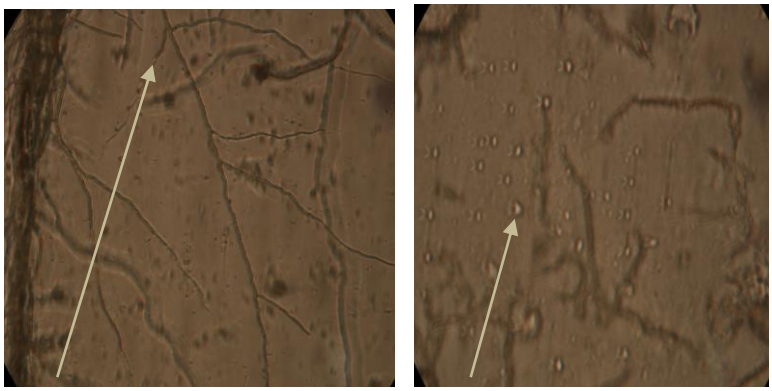
a. Conidiophores b. Fertile hair with long, straight, solitary and fertile apices c. Primary branches, branching towards tip

Figure 7 a, b, c, and d represent the morphology study of isolate No. 2 using light microscope

Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166



a. Sporangium b. Conidiophores c. Sporangium



d. Fertile hair with long, straight, solitary and fertile apices e. Conidia

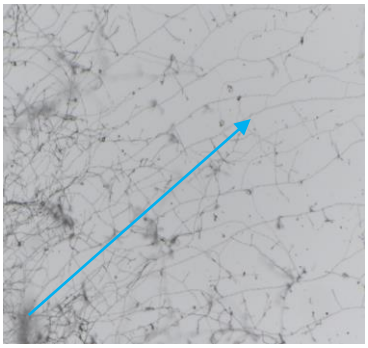
Figure 8 a, b, c, d, and e represent the morphology study of isolate No. 3 using light microscope

a. Spores branching b. Fertile hair with long, straight, solitary and fertile apices c. Primary branches, towards tip

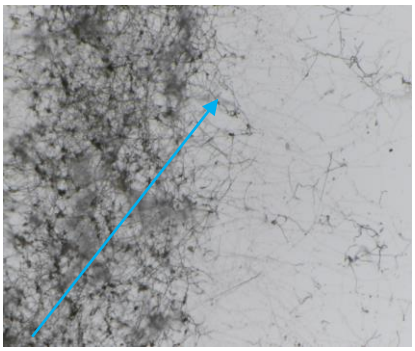
Figure 9 Figure plates a, b, and c represent the morphology study of isolate No. 1 using stereo microscope

Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166

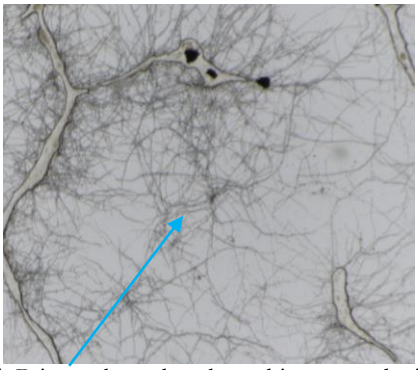
a. Fertile hair with long, straight, solitary and fertile apices



b. Fertile hair with long, straight, solitary apices

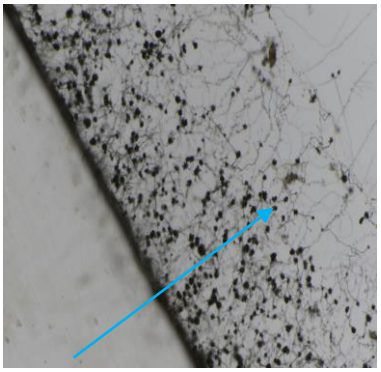


c. Sporangium

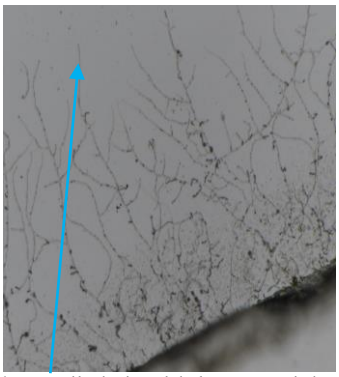


d. Primary branches, branching towards tip

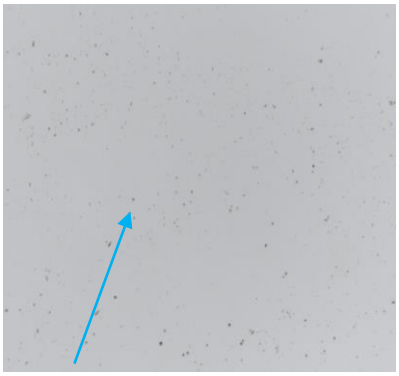
Figure 10 Figure plates a, b, c, and d represent the morphology study of isolate No. 2 using stereo microscope



a. Sporangium



b. Fertile hair with long, straight, solitary and fertile apices



c. Spores

Figure 11 Figure plates a, b, and c represents the morphology study of isolate No. 3 using stereo microscope

Cellulase production by Trichoderma sp.

All the three isolates of *Trichoderma* sp. showed cellulase production (Table 4). The production of cellulase enzyme was found to be more by isolate No. 1. There are reports on production of cellulase by *Trichoderma reesei* Cef19 (Kumar et al., 2014; Zhao et al., 2018). There is also a study on *Trichoderma harzianum* using cellulosic substrates (Delabona et al., 2015). Reports are there on cellulase enzyme production from *Trichoderma reesei* and *Aspergillus awamori* in submerged fermentation with rice straw (Naher et al., 2021). Cellulase production by *Aspergillus niger* by submerged and solid-state fermentation using coir waste as substrate has been studied (Mrudula & Murugammal, 2011).

Nep J Environ Sci (2023), 11(1), 11-17
https://doi.org/10.3126/njes.v11i1.49166

Table 4 Cellulase production by the three isolates of *Trichoderma* sp.

Isolates No.	Zone of clearance (cm)
1	1.4
2	1.3
3	0.9

Conclusions

The three *Trichoderma* sp. showed cellulase enzyme production which will have immense industrial applications which will be important. The *Trichoderma* sp. can also be used in the composting process to prepare the compost which can be used for the growth of crops/plants.

Conflicts of Interest: The author declares no conflicts of interest.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

References

da Silva Delabona, P., Lima, D.J., Robl, D., Rabelo, S.C., Farinas, C.S., & da Cruz Pradella, J.G. (2016). Enhanced cellulase production by *Trichoderma harzianum* by cultivation on glycerol followed by induction on cellulosic substrates. *Journal of Industrial Microbiology & Biotechnology*, 43(5), 617-626. doi 10.1007/s10295-016-1744-8.

Imran, M., Anwar, Z., Irshad, M., Asad, M., & Ashfaq, H. (2016). Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization in industry: A review. *Advances in Enzyme Research*, 4(2), 44-55. doi 10.4236/aer.2016.42005.

Kumar, R., Kumaran, M., Balashanmugam, P., Rebecca, I., Kumar, D., & Kalaichelvan, P. (2014). Production of cellulase enzyme by *Trichoderma reesei* Cef19 and its application in the production of bioethanol. *Pakistan Journal of Biological Sciences*, 17(5), 735-739. doi 10.3923/pjbs.2014.735.739.

Li, J.X., Zhang, F., Jiang, D.D., Li, J., Wang, F.L., Zhang, Z., Wang, W., & Zhao, X.Q. (2020). Diversity of cellulase-producing filamentous fungi from Tibet and transcriptomic analysis of a superior cellulase producer *Trichoderma harzianum* LZ117. *Frontiers in Microbiology,* 11, 1617. doi 10.3389/fmicb.2020.01617.

Mrudula, S., & Murugammal, R. (2011). Production of cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate. *Brazilian Journal of Microbiology*, 42(3), 1119-1127. doi 10.1590/S1517-838220110003000033.

Naher, L., Fatin, N., Sheikh, H., Azeez, A., Siddiquee, S., Zain, M., & Karim, R. (2021). Cellulase enzyme production from filamentous fungi *Trichoderma reesei* and *Aspergillus awamori* in submerged fermentation with rice straw. *Journal of Fungi,* 7(10), 868. doi 10.3390/jof7100868.

Teather, R., & Wood, P. (1982). Use of congo red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and Environmental Microbiology*, 43(4), 777-780. doi 10.1128/aem.43.4.777-780.1982.

Zhao, C., Liu, X., Zhan, T., & He, J. (2018). Production of cellulase by *Trichoderma reesei* from pretreated straw and furfural residues. *RSC Advances*, 8(63), 36233-36238. doi 10.1039/c8ra05936e.