THE CELLULASE ACTIVITY OF THE FUNGI THAT GROW ON THE BIO-BASED THERMAL INSULATION COMPOSITE MATERIALS

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Introduction

Bio-based thermal insulation materials are ecological materials made from a hemp, flax, jute, wood wastes. Such composites also consist the corn starch, the poly-L-lactide or natural lignin as the binders. Such materials are the environment-friendly, but under their exploitation the biodestructive factors arrise. At certain conditions like high humidity or the accumulation of moisture, such kind of materials may be susceptable to the microbial action which can lead to the changes of physical -chemical properties of materials, their destruction, and also the human health problems

Objectives

The biocomposite boards (2.5x2.5x1 cm) was spread with 5 ml of sterile water and left at the room temperature (22°C) in the sterile box for one week (Fig. 1a). The molds from these boards was inoculated by microbiology loops on the Petri dishes with solid PDA media [1] and was growing at 28°C for days (Fig.1b). Total DNA from pure cultures were isolated using a PureLink Microbiome DNA Purification Kit (Invitrogen)) in accordance with the manufacturer's instructions.

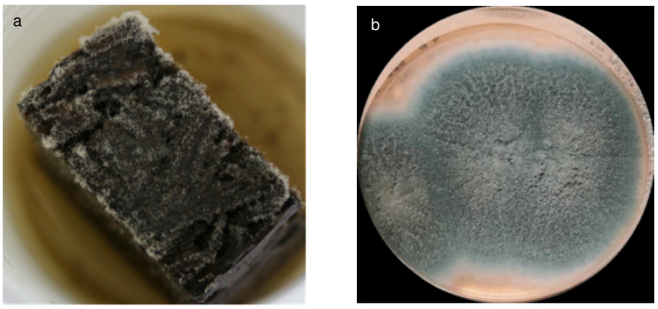

Fig. 1. Hemp shives biocomposite boards with the natural bider –lignin, after 1 week. The fungi o growing on the PDA media 28 day, at the temperature 28°C.

DNA amplification and identification

Using of the ribosomal ITS region primers ITS1F (5´ CTTGGTCATTTAGAGGAAGTAA-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) fungi belonging to *the Rhizopus oryzae, Sporothrix schenckii, Talaromyces pinophilus, Aspergillus fumigatus,* and *Trichoderma spp.* were identified. The quality of extracted DNA were determined using and 1% agarose gel-electrophoresis.

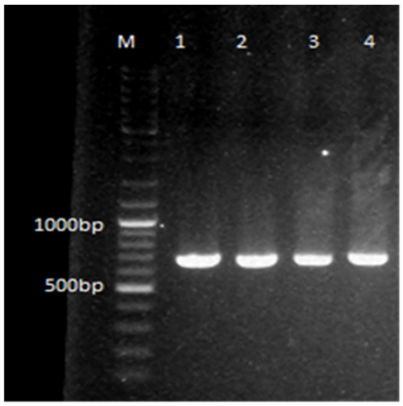

Fig. 2. PCR products of approximately 550 to 600 bp of ITS region. M- MW marker; 1 to 4 – PCR species, corresponding to the fungi depicted in Fig.1b

The PCR was performed in a total reaction of 50 µL, consisting of the kit DreamTaq green PCR Master Mix (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) and 1 µL of DNA template (5 ng) , according to the manu-facturer's instructions. PCR amplification was carried out by Bio-rad thermocycler using the following conditions: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min 30 s and 72 °C for 2 min. The final extension was carried out at 72 °C for 10 min. The PCR products were purified using a GeneJet PCR purification kit (Thermo Fisher Scientific Bal-tics, Vilnius, Lithuania). The ITS of rDNA regions as 5.8S rDNA was sequenced, and the obtained results was compared with known ITS sequences in NCBI GenBank. Our results revealed identity of *Aspergillus fumigatus,* represented in Fig.1b, in a lines 1 and 2.

Conclusions

In this work we attempted to isolate and identify the fungal species capable of growth on bio-based thermal isolation materials. As the starting material, fibre hemp shives samples with or without binding material, and with visible microbial activity. Cellulase activity assay were performed at the media from Czapek medium after 2 week, as carbon source was the sucrose and corn starche. The biobased composites medium were the most after 11 week, when the carbon source were the cellulose. It is know that some strains of *A.fumigatus* have the antimicrobial properties, in this research determined slightly antimicrobial activities.

The cellulase activity as determined by the Gram's iodine.

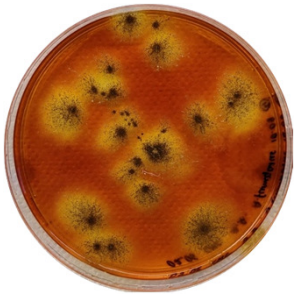

Fig. 3. Results of cellulase activity assay using Gram's iodine stained carboxymethylcellulose (CMC)-agar plates.

The obtained halos indicate that *A. fumigatus* has the cellulase activity.

The cellulase activities of *A.fumigatus* using the 3,5-dinitrosalicylic acid (DNS method)

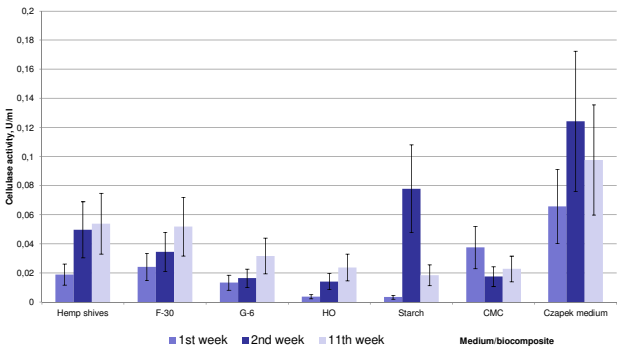

Fig. 4. Activity of cellulase enzymes using different biocomposites or medium for *A.fumigatus*.

Cellulase activity assay was performed using different biocomposites or growth media. Cultures were grown either for one, two, or eleven weeks before performing the DNS reactions. The highest cellulase activity was obtained in Czapek medium due to high amount of sucrose present. The variation of enzymatic activities is considered to be observed due to different amount of carbon sources in growth media such as sucrose, cellulose, or corn starch.

Antibacterial effects of *Aspergillus fumigatus*

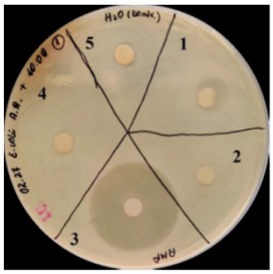

Fig. 5. Antibacterial activity tests.

Escherichia coli culture was grown on LB-agar. A cellulose paper discs were placed in each section [2]. Discs labeled 1, 2 and 4 were incubated with *A.fumigatus* culture, 3 – with solution of ampicillin, and 5 - distilled water as control. Small inhibition zones in 1, 2, and 4 regions allows to claim that *A.fumigatus* has the antimicrobial activity.

References

[1]Visagie, C.M.; Houbraken, J.; Frisvad, J.C.; Hong, S.B.; Klaassen, C.H.W.; Perrone, G.; Seifert, K.A.; Varga, J.; Yaguchi, T.; Samson, R.A.; 2014. Identification and nomenclature of the genus *Penicillium*. Studies in Mycology, 78(June, 2017): 343–371.
[2] Al-Fakih, A.A.; Almaqtri, W.Q.A. Overview on antibacterial metabolites from terrestrial *Aspergillus spp*. Mycology 2019, 10, 191–209.