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Patent application of antimicotic activity of a protein-rich aqueous extract of oyster mushroom (*Pleurotus ostreatus*) against *Aspergillus* spp. and *Penicillium* spp. moulds

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Figure 1. Photographs of the fruiting bodies of the oyster mushroom *Pleurotus ostreatus*.

BACKGROUND

Today, world face the challenge of an increasing number of infections caused by *Aspergillus* spp. moulds. Global mycological institutions are drawing attention to the selective pressure that has led to an increase in breakthrough infections also caused by dematiaceous moulds, *Penicillium* spp. Bioactive proteins and peptides derived from fruits, vegetables, meat or fish have great potential as functional foods or as substitutes for clinically used antimicrobials. In recent years, it has also been shown that the fungal kingdom could be a source of these compounds.

Pleurotus ostreatus is also known as the American oyster mushroom, oyster fungus, hiratake or pearl oyster mushroom. It belongs to the family *Pleurotaceae*. It is a common edible mushroom, widely used in gastronomy as a flavour-enhancing food additive, loaded with fibre, vitamins, minerals and other important nutrients, is an impressive source of antioxidants, has research-proven potential anti-tumour and anti-inflammatory effects. In addition, *P. ostreatus* mushrooms have been used in Traditional Chinese Medicine for thousands of years for their immune-boosting and anti-inflammatory properties, as well as in Ancient Rome and Greece.

The aim of this study was to investigate the bioactivity of the protein-rich aqueous extract derived from the oyster mushroom *Pleurotus ostreatus* and its hydrolysate against *Aspergillus* spp. and *Penicillium* spp. reference strains. The results of these studies have been notified to the Polish Patent Office (no. of the patent application: P.445190).

MATERIALS AND METHODS

FUNGAL MATERIAL Optimisation of fruiting body extraction, protein hydrolysis and matrix-assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) mass spectrometry for protein and peptide profiling of fungus samples (**Figure 1**) have been published previously[A].

MOULDS STRAINS The effect of extract and hydrolysate obtained by modified enzymatic hydrolysis from the oyster mushroom (*Pleurotus ostreatus*) on the growth of moulds of *Aspergillus* spp. and *Penicillium* spp. was studied. Moulds were cultured *in vitro* under aerobic conditions on solid Sabouraud medium at 35±2°C for 5-7 days. Followed reference strains from the American Type Culture Collection (ATCC) were used: *Aspergillus fumigatus* ATCC 46645, *Aspergillus niger* ATCC 16404, *Penicillium chrysogenum* ATCC 10106, from the collection of the Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland.

MINIMAL INHIBITORY CONCENTRATION (MIC) AND FUNGICIDAL CONCENTRATION (MFC) The activity of the tested extracts and hydrolysates obtained by enzymatic hydrolysis was assessed by **serial microdilution method** in a liquid medium, in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2022[B]. For this purpose, 96-well titer plates were used. A serial two-fold dilution to obtain the final concentrations of the tested extracts ranging from 0.156 to 32 mg/ml were performed. After 5 days of incubation at 35±2°C, the **MICs** were determined by microscope monitoring under the inverted microscope (Olympus DP22 automated inverted light). An appropriate positive (containing the inoculum of all microbial strains without the tested compounds) and negative controls (containing the tested extracts without the inoculum, including a sterile broth medium) were included on each microplate. The **MFC** was determined as the lowest drug concentration corresponding to 99.9% of the visual and microscopic (inverted optical microscope) reduction in fungal growth after incubation for 7-12 days at 35°C. The MFC/MIC ratio was estimated to investigate the fungicidal (>4) or fungistatic (≤4) effects of the extracts tested.

CONCLUSIONS

In conclusion, the protein- and peptide-rich oyster mushroom (*Pleurotus ostreatus*) extracts obtained are promising solutions with antifungal and antioxidant (presented earlier[A]) properties. The analysis of MIC and MFC values showing fungicidal activity against *Aspergillus* spp. and *Penicillium* spp. moulds can lead to the conclusion that aqueous extracts and hydrolysates of *P. ostreatus* can be an interesting alternative to known substances, including clinically used antifungal drugs.

After further research, the tested solutions may be used in the future as natural preservatives or anti-aging supplements due to their antioxidant properties, as well as to support the prevention and treatment of diseases caused by moulds, in particular *Aspergillus* spp. and *Penicillium* spp.[C]

RESULTS

As a result of the antifungal activity (Table 1), the protein- and peptide-rich oyster mushroom (*Pleurotus ostreatus*) extract tested showed the highest activity against the moulds (**Figure 2**), as follows: *A. fumigatus* ATCC 46645 and *A. niger* ATCC 16404 with MIC and MBC values of 1000 µg/ml and 8000 µg/ml (**Figure 3**), respectively, each showing fungicidal effect (MFC/MIC=1).

Table 1 Comparison of antifungal activity against *Aspergillus* spp. and *Penicillium* spp. of the tested oyster mushroom (*Pleurotus ostreatus*) extract obtained by the method of the research.

Strain	Studied extract			
	MIC [µg/ml]	MFC [µg/ml]	MFC/ MIC	Activity effect
Aspergillus fumigatus ATCC 46645	1000	1000	1	(+)
Aspergillus niger ATCC 16404	8000	8000	1	(+)
Penicillium chrysogenum ATCC 10106	8000	8000	1	(+)

Abbreviations: MIC – minimal inhibitory concentration; MFC – minimal fungicidal concentration; (+) - fungicide effect (MFC/MIC≤4)

Figure 2. Microscopic evaluation of the effect of a test extract of oyster mushroom (*Pleurotus ostreatus*) on reference strains of *Aspergillus* spp. and *Penicillium* spp. fungi

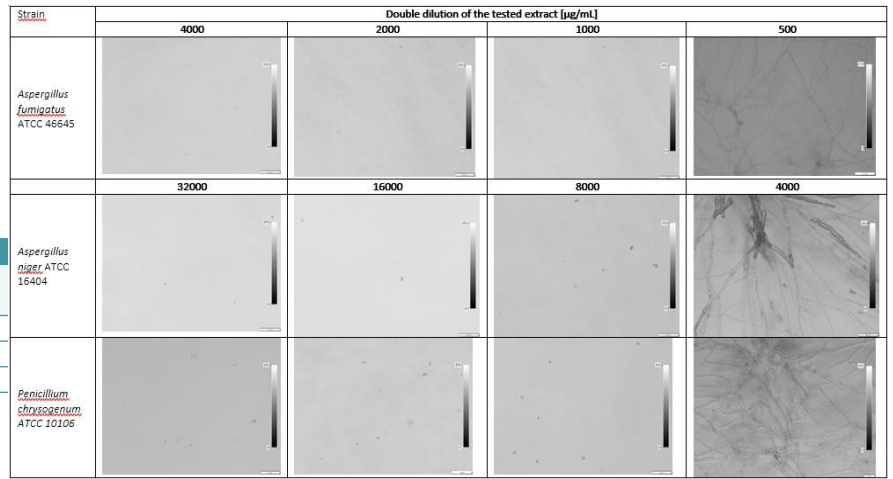
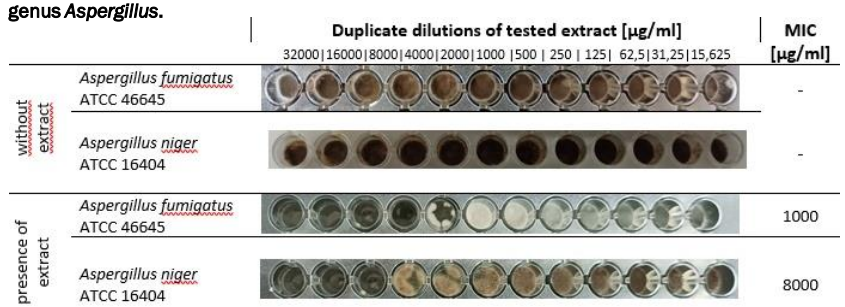


Figure 3. Evaluation of the MIC values of tested extract against ATCC strains of moulds of the genus *Aspergillus*.



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[A] Antioxidant and antimicrobial properties of an extract rich in proteins obtained from Trametes versicolor. Michalak K., S.Winiarczyk, Adaszek Ł., Kosikowska U., Andrzejczuk S., Garbacz K., Dobut A., Jarosz Ł., Czupryna W., Pietras-Ożga D. Journal of Veterinary Research 2023 T. 67 Nr 2 s. 209-218, DOI: 10.2478/jvetres-2023-0036

[B] EUCAST DEFINITIVE DOCUMENT E.DEF 9.4 2022. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds.

[C] A process for the preparation of a protein-rich aqueous extract of the oyster mushroom (Pleurotus ostreatus) and the use of the extract of Pleurotus ostreatus for assisting in the prevention and treatment of diseases caused by moulds, in particular Aspergillus and Penicillium. Aspergillus and Penicillium. Application No. P.445190