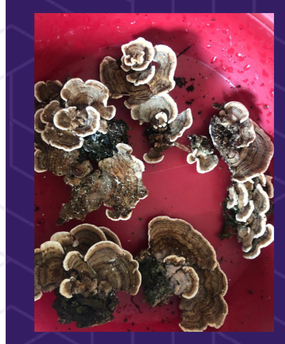

Anti-staphylococcal activity of a protein- and peptide-rich aqueous extract of *Trametes versicolor*

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Sylwia Andrzejczuk* 1, Martyna Kasela 1, Dorota Pietras-Ożga 2, Katarzyna Michalak 2, Małgorzata Tomczak 3, Julia Ćwiklak 3, Urszula Kosikowska 1

1 Department of Pharmaceutical Microbiology, Medical University of Lublin
2 Department of Epizootiology and Infectious Diseases Clinic, University of Life Sciences in Lublin, Poland
3 Students Research Group at the Department of Pharmaceutical Microbiology, Medical University of Lublin

sciforum-075193

sylwia.andrzejczuk@umlub.pl

Introduction

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 bioactive compounds. Studies of these organisms have indicated the presence of a significant proteins and peptides content. *Trametes versicolor* is a common fungal species of the family *Polyporaceae*, found throughout the world. Also known as Turkey tail, it usually grows on wood, making it an essential part of the ecosystem. It is beautiful to look at and relatively easy to identify.This mushroom has been used in folk medicine for thousands of years, while more recently it has been exploited as a source of polysaccharide K as an adjuvant immunotherapy for a variety of cancers. From the knowledge gained from our research, published earlier (1), it appears that all the samples tested showed a significant percentage of radical inhibition and it was shown that *T. versicolor* hydrolysate possessed stronger antioxidant properties. The analysis of the mass spectra for *T. versicolor* showed differences, especially in the range of higher peptide masses and for small proteins (5,000-10,000 Da and 10,000-20,000 Da).

In light of the above, the aim of this study was to investigate the bioactivity of an extract of the **lignicolous fungus *T. versicolor*** and its hydrolysate against both reference and clinical strains of *Staphylococcus* spp., including methicillin-resistant ones.The results of these studies have been reported to the Polish Patent Office (the patent application number: P.445189).

Materials and method

Fungus preparation 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 *Trametes versicolor* fungus samples (**Figure 1**) have been published previously (1). The extracts which are the subject of the invention were obtained by two methods, i.e. by using a freeze-drying process (TVLE, *Trametes versicolor* liophilised extract) or liquid nitrogen (TVE, *Trametes versicolor* extract) at the stage of preparation of the fungal material.

Bacterial strains The antimicrobial activity of the tested extracts was evaluated against either the reference staphylococcal strains from the American Type Culture Collection (ATCC), including: *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *S. aureus* ATCC 1707 MRSA, or eight methicillin-susceptible clinical *S. aureus* (MSSA) isolates of human origin.

Minimal Inhibitory Concentration (MIC) Assays was screened by using a microdilution broth method according to the protocols of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2). The sterile 96-well polystyrene microtitrate plates were prepared by adding 100 µL of the extracts tested diluted in an appropriate broth medium, per well, by a serial two-fold dilution to obtain the final concentrations of the tested extracts ranging from **0.001563 to 32 mg/mL**. After overnight incubation at 35±2◦C, the MICs were determined visually, as a result of an optically clear well, and at 590 nm by using a spectrophotometer microplate reader ELx800 (Biokom, Poland). An appropriate positive (containing the inoculum of all microbial strains without the tested compounds) and negative controls (containing the tested extracts without the bacterial inoculum, including a sterile broth medium) were included on each microplate.
Minimal bactericidal concentration (MBC) was obtained by a culturing 5 µL from each well and was visually recorded as the lowest concentration that established a predetermined reduction in bacteria (99.9%) measured in CFU/mL (colony forming units). The MBC values were defined as the lowest concentrations of the extract without the visual growth of microorganisms.

Results

The susceptibility of staphylococcal reference strains and clinical isolates towards extracts from *Trametes versicolor* fungus was shown in **Table 1**, based on their MIC and MBC values. The bactericidal or bacteriostatic effects of the extracts tested based on MBC/MIC ratio were also performed. TVLE extract showed the best activity against staphylococci, including *S. aureus* ATCC 43300 MRSA and *S. aureus* ATCC 29213, with MIC values of 500 µg/mL each.

Extracts TVLE treated by freeze-drying in the preparation phase had bactericidal properties with an MBC/MIC quotient ≤4, whereas those treated by freezing in liquid nitrogen TVE were bacteriostatic, regardless of strain.
A significant reduction in MIC values was achieved by the use of liquid nitrogen in the preparation of the fungal material to the range of 15.625 - 31.25 µg/mL for all reference staphylococcal strains, as well as methicillin-susceptible (MSSA) clinical ones (**Figure 2**).

Table 1. Anti-staphylococcal activity of tested protein- and peptide-rich extracts of *Trametes versicolor* obtained by the two modified methods of the invention

Bacterial strains		Tested extract					
		TVLE			TVE		
		MIC [µg/mL]	MBC [µg/mL]	MBC/MIC	MIC [µg/mL]	MBC [µg/mL]	MBC/MIC
reference	*Staphylococcus aureus* ATCC 29213	500	1000	2 (+)	15,625	1000	>4 (-)
	Staphylococcus aureus ATCC 25923	1000	2000	2 (+)	31,25	2000	>4 (-)
	Staphylococcus aureus ATCC 6538	1000	1000	1 (+)	15,625	1000	>4 (-)
	Staphylococcus aureus ATCC 43300 MRSA	500	1000	2 (+)	15,625	1000	>4 (-)
	Staphylococcus aureus ATCC 1707 MRSA	1000	1000	1 (+)	15,625	1000	>4 (-)
clinical	*S. aureus* isolate no. 1 MSSA	7,81	2000	>4 (-)	3,91	1000	>4 (-)
	S. aureus isolate no. 2 MSSA	500	1000	2 (+)	15,625	1000	>4 (-)
	S. aureus isolate no. 3 MSSA	1000	1000	1 (+)	31,25	2000	>4 (-)
	S. aureus isolate no. 4 MSSA	500	2000	4 (+)	15,625	1000	>4 (-)
	S. aureus isolate no. 5 MSSA	500	2000	4 (+)	15,625	1000	>4 (-)
	S. aureus isolate no. 6 MSSA	500	500	1 (+)	15,625	1000	>4 (-)
	S. aureus isolate no. 7 MSSA	500	2000	4 (+)	15,625	1000	>4 (-)
	S. aureus isolate no. 8 MSSA	500	1000	2 (+)	15,625	1000	>4 (-)

Abbreviations: MRSA - methicillin-resistant *Staphylococcus aureus*; MSSA - methicillin-susceptible *Staphylococcus aureus*; TVLE - *Trametes versicolor* extract after freeze-drying in the preparation of fungus; TVE - *Trametes versicolor* extract after the application of liquid nitrogen in the preparation of fungus; (+) - bactericidal effect; (-) - bacteriostatic effect

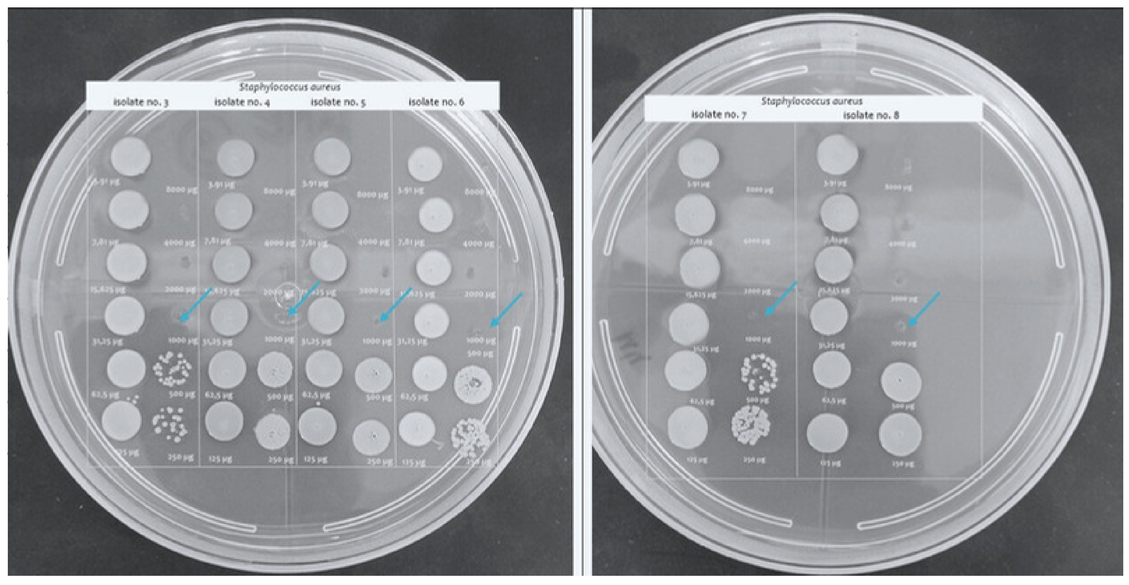

Figure 2. Evaluation of the MBC of a test aqueous extract of *Trametes versicolor* obtained by the modified process of the invention against six human origin clinical isolates of *Staphylococcus* spp.

Figure 1. Photographs of fruiting body of *Trametes versicolor* fungus

Conclusions

Trametes versicolor showed the investigated biological activities to an extent that suggests the usefulness of this fungus. By analysing the MIC and MBC values, we can conclude that water extract and hydrolysate of *T. versicolor* have a strong potential to be interesting alternatives to antibiotics due to their antimicrobial activity against bacterial strains such as staphylococci. In conclusion, the obtained protein-rich extract and its hydrolysate, obtained by using two different methods from the fungal material preparations, reported in the patent pending application (P.445189) (3), are promising solutions with strong antibacterial properties.

References

(1) **Antioxidant and antimicrobial properties of an extract rich in proteins obtained from *Trametes versicolor*. Michalak K., Winiarczyk S., 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**
(2) The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023. http://www.eucast.org
(3) Process for the preparation of an aqueous extract of Trametes versicolor rich in proteins and peptides, and use of the extract of *Trametes versicolor* for the prevention and treatment of diseases caused by staphylococci and streptococci. versicolor) to aid in the prevention and treatment of diseases caused by staphylococci and streptococci. Application No. P.445189.