Antimicrobial properties of shiitake mushrooms (Lentinula edodes)

Article *in* International journal of antimicrobial agents · June 2009

DOI: 10.1016/j.ijantimicag.2008.10.018 · Source: PubMed

4 authors, including:

Juluri Rao
Agri-Food and Biosciences Institute
77 PUBLICATIONS **1,122** CITATIONS
SEE PROFILE

Thomas J. Smyth
IT Sligo
67 PUBLICATIONS **1,225** CITATIONS
SEE PROFILE

Complementary Therapies in Clinical Practice 15 (2009) 5–7

Contents lists available at ScienceDirect

Complementary Therapies in Clinical Practice

journal homepage: www.elsevier.com/locate/ctnm

An examination of antibacterial and antifungal properties of constituents of Shiitake (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms

Rachel Hearst [a,b], David Nelson [a], Graham McCollum [a], B. Cherie Millar [c], Yasunori Maeda [c,d], Colin E. Goldsmith [c], Paul J. Rooney [c], Anne Loughrey [c], J.R. Rao [a], John E. Moore [c,d,*]

[a] Applied Plant Science Division, Agri-Food & Biosciences Institute, Newforge Lane, Belfast BT9 5PX, Northern Ireland, UK
[b] Grosvenor Grammar School, Cameronian Drive, Belfast BT5 6AX, Northern Ireland, UK
[c] Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Lisburn Road, Belfast BT9 7AD, Northern Ireland, UK
[d] School of Biomedical Sciences, Centre for Molecular Biosciences, University of Ulster, Cromore Road, Coleraine, Co. Londonderry, BT52 1SA, Northern Ireland, UK

Keywords:
Antibiotic resistance
Herbal remedy
Shiitake mushroom
Pseudomonas aeruginosa
Cystic fibrosis

A B S T R A C T

Background: Antibiotic agents have been in widespread and largely effective therapeutic use since their discovery in the 20th century. However, the emergence of multi-drug resistant pathogens now presents an increasing global challenge to both human and veterinary medicine. It is now widely acknowledged that there is a need to develop novel antimicrobial agents to minimize the threat of further antimicrobial resistance. With this in mind, a study was undertaken to examine the antimicrobial properties of aqueous extracts of 'exotic' Shiitake and Oyster mushrooms on a range of environmental and clinically important microorganisms.

Method: Several batches of Shiitake and oyster mushrooms were purchased fresh from a local supermarket and underwent aqueous extraction of potential antimicrobial components. After reconstitution, aqueous extracts were tested qualitatively against a panel of 29 bacterial and 10 fungal pathogens, for the demonstration of microbial inhibition.

Results: Our data quantitatively showed that Shiitake mushroom extract had extensive antimicrobial activity against 85% of the organisms it was tested on, including 50% of the yeast and mould species in the trial. This compared favourably with the results from both the Positive control (Ciprofloxacin) and Oyster mushroom, in terms of the number of species inhibited by the activity of the metabolite(s) inherent to the Shiitake mushroom.

Conclusions: This small scale study shows the potential antimicrobial effects of Shitake extracts, however further work to isolate and identify the active compound(s) now requires to be undertaken. Once these have been identified, suitable pharmaceutical delivery systems should be explored to allow concentrated extracts to be prepared and delivered optimally, rather than crude ingestion of raw material, which could promote further bacterial resistance.

1. Introduction

Since the discovery and exploitation of antibiotic agents in the 20th century, the targeted selective toxicity of such agents has ensured their widespread and largely effective use to combat infections. However, it has paradoxically resulted in the emergence and dissemination of multi-drug and even pan-resistant pathogens and this antimicrobial resistance in both medicine and agriculture is now recognized by the World Health Organisation (WHO), along with other various national authorities, as a major emerging problem of public health importance. Antibiotic resistance represents a significant challenge of global dimensions to human and veterinary medicine with the prospect of therapeutic failure for life-saving treatments now a reality. In order to minimize the potential development of further antimicrobial resistance "*The Copenhagen Recommendations: Report from the Invitational EU Conference on the Microbial Threat*" was published (http://www.im.dk/publikationer/micro98/index.htm), which outlined the need for the development of "*Novel principles for treating or preventing infections in humans and animals.*" Such an approach may thus be to examine the antimicrobial properties of 'exotic' mushrooms, such as Shiitake and Oyster, as novel sources of such agents, as well as the employment of such novel compounds, and thus limit the use of conventional antibiotics to cases of severe and life-threatening

* Corresponding author. Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast BT9 7AD, Northern Ireland, UK. Tel.: +44 (28) 9026 3554; fax: +44 (28) 9026 3991.
E-mail address: jemoore@niphl.dnet.co.uk (J.E. Moore).

1744-3881/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ctcp.2008.10.002

infections, thus minimizing the development of resistance to such agents.

Shiitake, *Lentinula edodes* is one of the most popular edible mushrooms in the world, production globally being second only to the button mushroom *Agaricus bisporus*.[1] Interest is increasing because of its high nutritional value and medicinal properties, traditionally acknowledged by oriental cultures, especially in China and Japan.[1,2] Compounds produced by *Lentinula* are attributed to have many functional properties, including a water soluble polysaccharide named 'lentinan', with antitumour and antiviral properties,[3–5] as well as antimicrobial potential.[6–9] Hypocholesterolemic[10] and hypoglycaemic[11] actions are also reported, via other compounds such as 'lentinacin' or 'lentysine'. *Lentinula* has shown no evidence of being acutely toxic, nor of having serious side effects.

Oyster mushroom (*Pleurotus ostreatus*) is also a common edible mushroom, which is now cultivated around the world for food. It is a saprotroph which acts as a primary decomposer on wood and is used industrially for mycoremediation, as well as a delicacy in Japan and China. A study has shown that the mushroom could lower blood glucose and cholesterol in diabetes patients.[12]

Given that there has been some evidence to date suggesting that these mushrooms may have some antimicrobial properties, it was the aim of the current study to perform a microbiological assessment of both antibacterial and antifungal properties of Shiitake, as well as Oyster mushrooms, against highly relevant bacterial clinical pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), as well as several members of the *Enterobacteriaeceae* and *Pseudomonas aeruginosa*, in addition to yeasts and filamentous fungi.

2. Materials and methods

Fresh Shiitake and oyster mushrooms were purchased from a local supermarket. For the extraction of metabolites from each of the fungi, fruiting body tissues were placed in an Edwards Supermodulyo Freeze drier, at −40 °C for a minimum of 48 h or until complete dryness occurred, which causes a 90% reduction in fresh weight. Following this dessication, the tissues were ground to a fine powder using a Braun Food Processor and a recorded weight of powder was then transferred to a suitably sized Schott bottle. Aqueous extracts were performed using 20× dry weight using sterile distilled water. All bottles were capped, thoroughly mixed to produce a slurry and stored in a refrigerator at 4 °C for 72 h, to elute the metabolites. Aliquots of the slurry were centrifuged at 9000 × g for 10 min using an Heraeus Biofuge Primo R centrifuge, following which, the supernatants were transferred to fresh containers. To reduce the volume of metabolite extracts that were thus produced, the supernatants were concentrated by freeze drying again to produce a powder. For assay purposes, a recorded weight of freeze dried powder was reconstituted with an equal weight of sterile 0.1% (w/v) peptone saline (CM0733, Oxoid Ltd., Basingstoke, UK) to give a known concentration for each extract solution.

Thirty-nine microorganisms, including 29 bacteria and 10 fungi, were challenged in this study to ascertain the antimicrobial properties of the two aqueous mushroom extracts. Of the bacterial isolates selected, 20 were Gram-negative organisms, which included seven genera, as well as nine Gram-positive organisms from three genera. Of the fungi examined, five were yeasts, with the remaining five being filamentous fungi, from five genera overall. These organisms are detailed in Table 1. Sterile 0.1% [w/v] peptone saline was used as a negative control and the antibiotic ciprofloxacin (5 μg disk) (MAST Diagnostics Ltd., Bootle, Merseyside, UK), was used as the positive control. The choice of ciprofloxacin was guided by the fact that it is a broad-spectrum antibiotic, thus having antibacterial properties for both Gram-positive and Gram-negative organisms.

In order to prepare the inocula for challenge, all organisms were cultured on Columbia Blood Agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood and incubated for 24 h at 37 °C (for bacterial and yeast organisms) and for 1 week (for filamentous fungi). Under aseptic conditions, dilutions of each isolate were prepared individually in 0.1% [w/v] peptone saline (PS) (Oxoid CM0733), equating to a 0.5 McFarland Standard (approximately 10^6 colony forming units (cfu) per ml) which was inoculated on to fresh Mueller–Hinton Agar (Oxoid CM0337), by means of a sterile cotton swab. To this, fresh extracts (10 μl) were added and the inoculum allowed to dry prior to incubation, as detailed above. Following this, plates were examined visually and any inhibition noted and its diameter measured (mm) and recorded.

Table 1
Diameter (mm) of zone of inhibition produced on a range of environmental and clinically important microorganisms using Shiitake (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms. 0.1% (w/v) peptone saline acted as the negative control and the antibiotic, ciprofloxacin (5 μg disk) acted as the positive control.

Microorganism	Mushroom		−ve control	+ve control
	Shiitake	Oyster	0.1% PS[a]	Cipro[b]
Bacteria				
Bacillis cereus NCTC 7464	12	5		24
Bacillis subtilis NCTC 10400 (NCIMB 8054)	11	7		33
Bacillus pumilis	14			26
Cupriavidis sp	15			27
E. coli NCTC 25922	12			29
E. coli NCTC 9001	9			27
E. coli 0157 NCTC 12900	10			27
Enterobacter/Klebsiella sp.	10			34
Enterococcus faecalis NCTC 775	8			15
Klebsiella aerogenes NCTC 9528	9			24
Klebsiella pneumoniae 700603	8			23
Listeria monocytogenes NCTC 11994	11			28
Pseudomonas aeruginosa NCTC 1662	9			25
Pseudomonas aeruginosa NCTC 27853	9			28
Pseudomonas sp 1	86	20		
Pseudomonas sp 20	8			18
Pseudomonas sp 3	9			
Pseudomonas sp 6	92			20
Salmonella poona NCTC 4840	9			26
Serratia marcescens	10			26
Serratia/Rahnella sp.	9			40
Staphylococcus aureus (MSSA) 25923	12			25
Staphylococcus aureus (MRSA) 43300	12			19
Staphylococcus epidermidis NCTC 11047				
Staphylococcus epidermidis NCTC 14990	11			28
Staphylococcus sp. NCTC 6571	12			27
Wildtype hand bacteria 1	10			23
Wildtype hand bacteria 2	12			24
Wildtype hand bacteria 3	14			24
Yeasts and filamentous fungi				
Aspergillus flavus QC 6658				ND
Aspergillus fumigatus 27.5	20			ND
Aspergillus niger 27.5	10			ND
Candida albicans				ND
Candida glabrata ATCC 2001				ND
Candida krusei ATCC 6258 27.5	15			ND
Candida parapsilosis ATCC 22019	11			ND
Exophiala (*Wangiella*) *dermatitidis* QC 7895				ND
Penicillium sp. QC 743275				ND
Scedosporium apiospermum QC 7870	12			ND

Blank = a zone of 0 mm inhibition reflecting no inhibition of growth and is therefore of equal value to the negative control (0.1% PS); ND = not determined.
[a] 0.1% PS = 0.1% (w/v) sterile peptone saline solution.
[b] Cipro, 5 μg ciprofloxacin disk.

3. Results and discussion

The antimicrobial activity of the two aqueous mushroom extracts and control extracts against 39 bacterial, yeast and fungal pathogens is shown in Table 1.

The Shiitake mushroom extract (1 mg/μl) demonstrated antimicrobial activity against 33/39 (84.6%) of these microorganisms (zone of inhibition range: 8–92 mm; mean = 15.7 mm). Five out of the ten yeast and mould species were inhibited. Some 26/39 (66.6%) organisms gave a zone of inhibition (range: 15–40 mm zone of inhibition; mean = 25.2 mm) when tested against the positive control (ciprofloxacin 5 μg disk). There was complete microbial confluence at the site of inoculation of the negative control (0.1% PS). The Oyster mushroom extract at this concentration showed activity against only 3/39 (7.6%) of the same range of pathogens (zone of inhibition range: 5–20 mm; mean = 10.7 mm), but did not inhibit the growth of any of the ten yeast and mould species examined.

One isolate, namely the coagulase-negative staphylococci, *Staphylococcus epidermidis*, was totally resistant to all antimicrobial agents tested, including the mushroom extracts and ciprofloxacin. The shiitake extract demonstrated good activity against the MRSA isolate tested (*S. aureus* (MRSA) 43300), in the manner in which honey has previously been utilized.[13] On three occasions, namely with the *Pseudomonas* sp isolates 1, 3 and 6, the shiitake extract was significantly more antibacterial than ciprofloxacin (positive control), whereby it gave markedly greater zones of inhibition. This is the first report of extracts of shiitake mushroom displaying anti-pseudomonal properties *in vitro* and is of important clinical significance, as *P. aeruginosa* is emerging as a major aetiological of nosocomial infection, particularly within patient populations with cystic fibrosis (CF). Prolonged exposure from early childhood in CF patients to conventional anti-pseudomonal antibiotics, such as ceftazidime and the β-lactams, have allowed the emergence of multi- and pan-resistant organisms, which are very difficult to treat clinically. The reporting of novel anti-pseudomonal activity with a natural compound is exciting and requires additional exploration and follow-up. In patients with cystic fibrosis, *P. aeruginosa* infection originates when these patients initially become colonized with this organism, usually from an environmental source, e.g., from water or a water related activity. Generally the first isolated of *P. aeruginosa* is sensitive to most antibiotics, if this organism has been acquired from the environment. Current antibiotic treatment regimes for first isolates of *P. aeruginosa* employ the anti-pseudomonal activity of ciprofloxacin in combination with nebulised colomycin. Thus, activity of Shitake extracts against *P. aeruginosa* is an important comparator, for potential use in this clinical setting.

This small scale study shows the potential antimicrobial effects of Shitake extracts, however further work to isolate and identify the active compound(s) now requires to be undertaken. Once these have been identified, suitable pharmaceutical delivery systems should be explored to allow concentrated extracts to be prepared and delivered optimally, rather than crude ingestion of raw material, which could promote further bacterial resistance. The efficacy of any resulting treatment regimen should subsequently be proven with well designed randomised control trials.

Acknowledgements

Rachel Hearst was supported by a Nuffield Science Bursary, administered by Sentinus. JEM is supported by an ID-RRG Project Grant (RRG 9.9) awarded by the HPSSNI Research & Development Office.

References

1. Wasser SP. Encyclopedia of dietary supplements 2005:653–4. doi:10.1081/E-EDS-120024880.
2. Chang ST, Buswell JA. Mushroom nutriceuticals. *World J Microbiol Biotechnol* 1996;**12**:473–6.
3. Maeda YY, Takahama S, Yonekawa H. Four dominant loci for the vascular responses by the antitumor polysaccharide, lentinan. *Immunogenetics* 1998;**47**:159–65.
4. Ng ML, Yap AT. Inhibition of human colon carcinoma development by lentinan from shiitake mushrooms (*Lentinus edodes*). *J Altern Complement Med* 2002:581–9.
5. Ngai PH, Ng TB. Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. *Life Sci* 2003;**73**:3363–74.
6. Komemushi S, Yamamoto Y, Fujita T. Antimicrobial substance produced by *Lentinus edodes*. *J Antibacterial Antifungal Agents* 1995;**23**:81–6.
7. Komemushi S, Yamamoto Y, Fujita TT. Purification and identification of antimicrobial substances produced by *Lentinus edodes*. *J Antibacterial Antifungal Agents* 1996;**24**:21–5.
8. Hatvani N. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. *Int J Antimicrob Agents* 2001;**17**:71–4.
9. Ishikawa NK, Kasuya MCM, Vanetti MCD. Antibacterial activity of Lentinula edodes. *Braz J Microbiol* 2001;**32**:206–10.
10. Sugiyama K, Arashi T, Yamakawa A. Eritadenine-induced alteration of hepatic phospholipid metabolism in relation to its hypocholesterolemic action in rats. *Nutrit Biochem* 1995;**6**:80–7.
11. Yang BK, Kim DH, Jeong SC, Das S, Choi YS Shin JS, Lee SC, et al. Hypoglycemic effect of a *Lentinus edodes* exo-polymer produced from a submerged mycelial culture. *Biosci Biotechnol Biochem* 2002;**66**:937–42.
12. Kathatun K, Mahtab H, Khanam PA, Sayeed MA, Khan KA. Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects. *Mymensingh Med J* 2007;**16**:94–9.
13. Maeda Y, Loughrey A, Earle JA, Millar BC, Rao JR, Kearns A, et al. Antibacterial activity of honey against community associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *Complement Ther Clin Pract* 2007;**14**:77–82.