Evaluation of Antimicrobial Activity of Fourteen Medicinal Basidiomycete Fungi against Yeast
and Antibiotic Resistant *Escherichia coli*

By

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Abstract

Antibiotic resistance of pathogenic microorganisms is a growing concern to society and treatments require the discovery of new antimicrobial compounds. This study evaluated fourteen different edible and/or medicinal mushroom species for antimicrobial activity, *Lentinula edodes*, *Agaricus bisporus*, *Clitocybe nuda*, *Armillaria mellea sensu stricto*, *Armillaria solidipes*, *Armillaria gallica*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Laetiporus sulphureus*, *Ganoderma lucidum*, *Ganoderma tsugae*, *Grifola frondosa*, *Hypsizygus tessellatus* and *Boletus edulis*. Growth inhibition of the yeast, *Saccharomyces cerevisiae*, and of ciprofloxacin-resistant strains of *Escherichia coli* was examined using bioassays and by Minimum Inhibitory Concentration (MIC) with alcohol extracts of mushroom mycelia and culture broth. Mycelia used for extract preparations were grown in Potato Dextrose Broth (PDB) with or without induction using a) low-nutrient medium, b) spent *Escherichia coli* filtrate, or c) 5-azacytidine. Several extracts resulted in a reduction in bacterial and/or yeast growth, although full inhibition was observed with only a few extracts. Fungi that were most effective as inhibitors in bioassays were *L. edodes*, *C. nuda*, *A. bisporus*, *A. solidipes*, *A. mellea (s. s.)* and *L. sulphureus*. The most active ethanol extracts were from *L. edodes*, *C. nuda*, *A. solidipes* and *L. sulphureus*. This study confirms that basidiomycetes may provide new, useful antimicrobials. Fungi with antibacterial and/or antifungal activity may be useful as a food therapy as well as leads in drug discovery.

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Table of contents

Abstract	ii
Acknowledgements	iii
Table of contents.....	iv
List of Figures and Tables.....	vi
SECTION I: Introduction.....	7
Antibiotic Resistance	7
Description of Basidiomycete Antagonists.....	10
<i>Lentinula edodes</i>	10
<i>Pleurotus</i> spp	12
<i>Laetiporus sulphureus</i>	13
<i>Agaricus bisporus</i>	14
<i>Armillaria</i> spp.....	15
<i>Clitocybe nuda</i>	15
<i>Hypsizygus tessellatus</i>	16
<i>Ganoderma</i> spp.....	17
<i>Grifola frondosa</i>	18
<i>Boletus edulis</i>	19
Antimicrobial Activity Detection.....	20
SECTION II: Materials and Methods	24
Growth of mycelia.....	24
Induction Treatments	25
Crude Extractions	27
Top Agar Susceptibility Bioassay	27
Confirmation of Ciprofloxacin Resistance in <i>E. coli</i> mutants	28
Minimum Inhibitory Concentration Assays	28
Monitoring for Biocidal and Biostatic Activity.....	29
Spawn-on-Logs.....	30
SECTION III: Results	32
Characteristics of Basidiomycete (Antagonist) Fungi Used in this Study	32
<i>Lentinula edodes</i>	32
<i>Pleurotus</i> spp.	32
<i>Laetiporus sulphureus</i>	33
<i>Agaricus bisporus</i>	33
<i>Armillaria</i> spp.	34
<i>Clitocybe nuda</i>	35
<i>Hypsizygus tessellatus</i>	36
<i>Ganoderma</i> spp.....	36
<i>Grifola frondosa</i>	37
<i>Boletus edulis</i>	38
Confirmation of Resistance/Sensitivity to Ciprofloxacin by <i>E. coli</i> strains	43
Top Agar Bioassays	43
Minimum Inhibitory Concentration Tests.....	46
Relationship between Top Agar and Minimum Inhibitory Concentration Tests	59

<i>Biostatic vs biocidal activity of extracts</i>	61
SECTION IV: Discussion	64
SECTION V: References	76
Appendix I- Plate Outlines	91
Appendix II-MIC Results Summaries	93
Appendix III-Induced vs Un-Induced Figures	107

List of Figures and Tables

Table 1. Species List.....	23
Figure 1. Spawn-on-Log.....	29
Figure 2. <i>Lentinula edodes</i>	39
Figure 3. <i>Pleurotus spp</i>	38
Figure 4. <i>Laetiporus sulphureus</i>	39
Figure 5. <i>Agaricus bisporus</i>	40
Figure 6. <i>Armillaria spp</i>	40
Figure 7. <i>Clitocybe nuda</i>	41
Figure 8. <i>Hypsizygus tessellatus</i>	41
Figure 9. <i>Ganoderma spp</i>	42
Figure 10. <i>Grifola frondosa</i>	42
Figure 11. <i>Boletus edulis</i>	41
Figure 12. CipR-mutant inoculations	42
Figure 13. Top Agar example of <i>Lentinula edodes</i>	43
Table 2. Inhibition zone diameters	45
Table 3. Final concentrations of crude extracts	48
Figure 14. MIC ₅₀ comparisons of mycelia and media extracts from 'induced' cultures.....	50
Figure 15. MIC ₉₀ comparison of mycelia and media extracts from 'induced' cultures	51
Figure 16. General comparison of MIC ₅₀ -MIC ₉₀ values and MIC ₅₀ - MIC ₁₀₀ values	52
Figure 17. MIC ₅₀ of mycelial and media extracts of 'un-induced' cultures.....	55
Figure 18. MIC ₉₀ of mycelial and media extracts of 'un-induced' cultures.....	56
Figure 19. MIC ₁₀₀ values of fungal extracts against <i>E. coli</i>	57
Figure 20. Effect of culture extracts against <i>S. cerevisiae</i>	58
Figure 21. Correlation between mean inhibition diameter and MIC ₅₀	60
Table 4. Mechanism of inhibitory action of extracts	63

SECTION I: Introduction

Antibiotic Resistance

Bacterial resistance to antibiotics has been a concern almost since antibiotics were first introduced into the market. Only a year after the widespread use of penicillin, multiple strains of *Staphylococcus aureus* were discovered to be resistant to this drug (Alanis, 2005). Since then, many bacteria previously susceptible to antibiotics have been found to be resistant to penicillin, and then some became resistant to multiple antibiotics. To date, at least one mechanism of resistance has been documented to most classes of antibiotics, including β -lactams, fluoroquinolones, aminoglycosides, sulfonamides, extended spectrum β -lactamases and even macrolides (Lin *et al.* 2015; Blair *et al.* 2015; Wierzbowski *et al.* 2007; Alanis, 2005). In addition, bacteria that have acquired resistance mechanisms to multiple antibiotic classes has become a growing issue, whether by general defense mechanism that renders the bacteria resistant to multiple compounds, or by a specific defense mechanism that blocks the target of certain compounds. These multidrug resistant bacteria (MDR) are becoming harder to diagnose and treat with their progressive acquisition of mutations conferring resistance. MDR bacteria of concern include *Mycobacterium tuberculosis* [causal agent of Tuberculosis (TB)], strains of which have become resistant to all major TB drugs (American Academy of Microbiology, 2009), Methicillin-resistant *Staphylococcus aureus*, which is resistant to multiple antibiotic classes (Nikaido, 2009), and naturally resistant *Burkholderia cepacia*, a causal agent of pneumonia (American Academy of Microbiology, 2009). Treatment of multi-drug resistant bacteria such as these can be difficult, lengthy and expensive, and infections by MDR microbes may be especially detrimental to immuno-compromised patients.

Among the hypotheses explaining the increased incidence of antibiotic resistance is the inappropriate use of antibiotics in animal feed (Fey *et al*, 2000; Wegener, 1999), where healthy animals are treated with antibiotics as a method of prophylaxis and to promote growth, with the hopes of raising uninfected animals that are safe for consumption. In another perspective, The American Academy of Microbiology attributes antibiotic resistance simply to Darwinian evolution, where natural selection and competition between microorganisms is promoted by the use, appropriate or not, of antibiotics (American Academy of Microbiology, 2009). In other words, resistance is a naturally occurring phenomenon that is unavoidable, and further promoted through the widespread use of antibiotics.

In many cases the mutations responsible for antibiotic resistance are well characterized. For example, *Escherichia coli* strains that are resistant to fluoroquinolone drugs, such as ciprofloxacin, have mutations in one or both the *marR* and *gyrA* genes. The *gyrA* mutations result in the alteration of the topoisomerase II subunit, reducing the affinity of fluoroquinolone drugs to their primary binding site in the topoisomerase II subunit. The *marR* mutations alter the function of a repressor of the *marRAB* operon, that alters the cell's efflux pumps and outer membrane pores (Alekhun and Levy, 1999; Sulavik *et al.* 1995). Mutations in *marR* can result in decreased antibiotic accumulation in the cell, resulting in resistance against the drug.

The study of resistance to fluoroquinolone drugs in *E. coli* has practical applications. First, *E. coli* is a model organism with well-developed genetic resources available that is amenable and relatively safe for laboratory research. Second, while *E. coli* is naturally found in the digestive tracts of healthy humans and other mammals, infections by some strains such as O517:H7 can cause bloody diarrhoea, severe anemia and kidney failure. Other harmful strains

can lead to urinary tract infection (American Public Health Association, 2008), making resistance in *E. coli* a medical concern. Finally, fluoroquinolone resistant *E. coli* mutants can serve as a model for studying resistance in other pathogenic bacteria, such as *Pseudomonas aeruginosa*, a harmful opportunistic bacterium, commonly found in secondary infections after a primary infection has taken place (i.e. nosocomial infections) (Mesaros *et al.* 2007). *P. aeruginosa* has acquired resistance towards several antibiotic classes, and fluoroquinolone resistance is often associated with mutations in *gyrA*, mentioned above. Understanding and addressing the resistance in the mutant *E. coli*, and identifying antimicrobials that are effective in inhibiting growth of resistant strains, can therefore aid the research and treatments of *P. aeruginosa* and other pathogenic bacteria.

Similarly, fungi that are pathogenic on animals and are resistant to commercially available antifungals are also showing up in clinical settings (Sanglard, 2002). Antifungals can be grouped into several classes based on their site of action, such as: azoles, which inhibit the synthesis of the fungal sterol ergosterol; polyenes, which interact with fungal membrane sterols; echinocandins that inhibit the synthesis of glucans in the cell wall; and 5-fluorocytosine, a pyrimidine analogue, which inhibits macromolecular synthesis (Sanglard, 2002; Ghannoum and Rice, 1999). Mechanisms of resistance have been reported for all classes of antifungal agents but have been mainly documented for the commonly used polyenes and azole antifungals. The yeast *Saccharomyces cerevisiae* is a good model for fungal pathogens such as *Candida* spp. and *Cryptococcus* spp., since it is an excellent organism for research on genetics, biochemistry and cell biology (Sanglard, 2002).

With the increasing incidence of antibiotic resistance by infectious disease organisms, it

has become imperative to identify new antimicrobial candidates. The naturally occurring antimicrobial compounds in plants and fungi can help lead to the discovery of new antibiotics for use against resistant microbial pathogens. These eukaryotic organisms naturally produce secondary metabolites as a defense against pathogens and competitors, and as anti-feedants. These compounds can be toxins and/or distasteful and may possess antimicrobial characteristics. As a group, plants and fungi produce many biologically active compounds so there is large source of products to explore for antimicrobial activity. Common examples of antimicrobials from natural sources include flavonoids, phenols, sterols, alkaloids, etc. Specifically, fungi provide an interesting area of study as many mushroom species contain a diversity of biomolecules with nutritional and/or medicinal value (Kalac, 2009). Chinese culture has been using mushrooms for their medicinal properties for thousands of years for the treatment of ailments and as part of a healthy diet to boost the immune system (Poucheret *et al.* 2006; Fons *et al.* 2005; Ying *et al.* 1987). Mushrooms can contain various secondary metabolites (Schulz *et al.* 2002), many being antibacterial. New antimicrobial candidates can be derived from these medicinal mushrooms that may help address the issue of antibiotic resistance. Indeed, previous studies have characterized antimicrobial activity in various fungal species, with some studies having identified active chemical compounds. My study evaluates the antimicrobial activity of the fourteen species of mushroom-forming, edible fungi described below.

Description of Basidiomycete Antagonists

***Lentinula edodes*.** *Lentinula edodes* (common name 'Shiitake') is commonly used in Traditional Chinese Medicine (TCM) as a food therapy for its immunomodulatory properties and is one of

the most commonly studied mushrooms (Poucheret *et al.* 2006; Chang, 1996). *L. edodes* is known to produce the compounds, lentinan, KS-2, and eritadenine. Lentinan is the most studied of these and has been found to have anticancer/antitumour effects that are believed to be a result of immunopotentiality. Lentinan has been used in many clinical trials and approved in treating gastric cancer as an adjunct therapy for patients undergoing chemotherapy (Mayell, 2001; Matilla *et al.* 2000; Chang, 1996). It was found that survival rates of cancer patients improved in patients undergoing chemotherapy-lentinan adjunct therapy versus with chemotherapy alone (Poucheret *et al.*, 2006; Chang, 1996). Lentinan is also believed to have antibacterial, antiviral and antiparasitic activity by improving host defense. In clinical applications, lentinan is part of an HIV and AIDS treatment and was found to improve liver function in a Hepatitis B clinical case study (Poucheret *et al.* 2006; Tochikura *et al.* 1998; Amagasse, 1987; Chang, 1996). Of the two less studied compounds, KS-2 is a polysaccharide that was shown to suppress tumour growth in mice, and induced interferon production in cancer patients; its antitumour activity continues to be studied (Jones, 1995). Eritadenine is a nucleic acid found to lower serum levels of cholesterol and lipid concentrations in rodents (Chang, 1996, Chibata *et al.*, 1969).

L. edodes extracts have also been studied for antimicrobial activity. A review by Alves *et al.* (2012) summarizes antimicrobial activity of various basidiomycetes and outlines the activity of *L. edodes* against a number of Gram-positive and Gram-negative bacteria. Several extracts were found to be active towards specific bacteria: Ishikawa *et al.* (2001) reported the inhibitory activity of ethyl acetate extracts of *L. edodes* against *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, and *S. epidermidis*. The aqueous extract was active against methicillin-resistant

Staphylococcus aureus (MRSA) (Hearst *et al.* 2009; Hur *et al.* 2004); the chloroform extract was found to inhibit growth of *Streptococcus pyogenes* (Hatvani, 2001). Antimicrobial compounds isolated from *L. edodes* include oxalic acid (Bender *et al.* 2003) and lenthionine (Hatvani, 2001; Yasumoto *et al.* 1971; Morita, 1967). *L. edodes* extracts also showed bactericidal effects on *Streptococcus mutans* and *Prevotella intermedia*, indicating these extracts may have applications in oral health (Alves *et al.* 2012; Hirasawa *et al.* 1999). Alves *et al.* (2012) reported fruit-body extracts of *L. edodes* showed inhibitory activity against *E. coli*, although Hatvani (2011) reported mycelial extract of *L. edodes* had no inhibitory effect on *E. coli*.

***Pleurotus* spp.** The *Pleurotus* spp. evaluated in my study are *Pleurotus ostreatus* (common name 'Oyster Mushroom') and *Pleurotus eryngii* (common name 'King Oyster'). These two edible mushrooms have nutritional value due to their high microelement, fibre and protein content, and are low in fat content (Mishra *et al.* 2013; Mori *et al.* 2008). In addition, they are known to have biological activities due to their polysaccharide content. *P. ostreatus* contains a diverse composition of antioxidants as well, including ascorbic acid, vitamin C, tocopherol, vitamin E, and β -carotene (Iwalokun *et al.* 2007; Elmastas *et al.* 2007; Yang *et al.* 2002). *P. eryngii* is noted for having a high phenolic content (Mishra *et al.* 2013). Antimicrobial properties have been investigated for both fungi; *P. ostreatus* showed broad spectrum antibacterial activity, including against *E. coli*, and antifungal activity (Sharma *et al.* 2014; Alves *et al.* 2012; Hearst *et al.* 2009; Iwalokun *et al.* 2007), and *P. eryngii* inhibited growth of *E. coli* and *S. aureus* (Alves *et al.* 2012). Extractions differed in studies of antibacterial activity in *P. ostreatus*; aqueous extracts were only active against three of 39 bacterial species tested, *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas* sp. (Hearst *et al.* 2009). Ethanol extracts were found to have

broader activity against both Gram-positive and Gram-negative bacteria (Alves *et al.* 2012), although in a comparative study against *L. edodes*, *P. ostreatus* was not as effective as an antimicrobial (Iwalokun *et al.* 2007). Different extracts of *P. eryngii* also resulted in inhibition of different bacterial species. For example, acetone extracts of *P. eryngii* did not demonstrate antimicrobial effects against *B. megaterium*, *K. pneumoniae*, or *S. aureus*, but inhibited growth of *M. luteus* and *P. denitrificans*, while the ethyl acetate extracts of *P. eryngii* showed no activity against *M. luteus* and *P. denitrificans* but did show activity against *B. megaterium*, *K. pneumoniae*, and *S. aureus* (Akyuz and Kirbag, 2009; Uzen *et al.* 2004). Anticancer and antitumour agents were also investigated in both of these species; a study of the polysaccharide (PEPw) isolated and purified from *P. eryngii* potentiated immunological function of tumor-bearing mice (Yang *et al.* 2013) and polysaccharide (POP-1) and proteoglycans from the mycelia of *P. ostreatus* were reported to be immunomodulating agents with potential antitumour activity demonstrated in mice models (Tong *et al.* 2009; Sarangi *et al.* 2006), although the mechanisms for these activities are still unclear.

***Laetiporus sulphureus*.** Another medicinal mushroom, *Laetiporus sulphureus* (common names include 'Sulphur Shelf' and 'Chicken-of-the-Woods'), is a fungus that has long been used as a source of antioxidants, antimicrobials and as an immunostimulant (Sinanoglou *et al.* 2015; Turkoglu *et al.* 2007). Both hexane and chloroform extracts of *L. sulphureus* demonstrated antifungal and antibacterial properties, with the hexane extracts demonstrating more effective results (Sinanoglou *et al.* 2015). Ethanol extracts inhibited the growth of *Bacillus subtilis*, *B. cereus*, *Micrococcus luteus*, and *M. flavus*. Ethanol extracts inhibited the growth of *E. coli* but were overall less effective on Gram-negative bacteria (Alves *et al.* 2012; Turkoglu *et al.* 2007).

Turkoglu *et al.* (2007) also described the antioxidant activity of *L. sulphureus*. It has a varied composition of polyphenols and flavonoids whereby 320 µg of ethanol extract had equivalent activity to 40 µg α-tocopherol. Mlinaric *et al.* (2005) also investigated the ability of *L. sulphureus* to inhibit HIV-1 reverse transcriptase and found that methanolic extracts inhibited more than 40% HIV-1 RT *in vitro*.

Agaricus bisporus. This basidiomycete (common names include 'Button Mushroom', 'Cremini' and 'Portobello') has been often cited for antibacterial and antioxidant activities. Numerous extracts of *A. bisporus* are reported to have antibacterial activity against several Gram-positive and Gram-negative bacteria, including *B. subtilis* at MIC=5 µg /ml, lower than the standard ampicillin (MIC= 12.5µg /ml), and *E. coli* (Sharma *et al.* 2014; Alves *et al.* 2012; Barros *et al.* 2008). In fact, many *Agaricus* species have been reported to have antibacterial activity; methanolic extracts of *Agaricus bitorquis* and *Agaricus essettei* showed inhibitory effects on several Gram-positive bacteria (Ozturk *et al.* 2011) and methanolic extracts of *Agaricus silvicola* showed antibacterial activity against *Bacillus cereus*, *Bacillus subtilis* and against *Staphylococcus aureus* (Alves *et al.* 2012; Barros *et al.* 2008). To date, active antibacterial compounds of *A. bisporus* have not been identified, to my knowledge. Elmastas *et al.* (2007) recorded antioxidant activity in the methanolic extracts of *A. bisporus*, wherein there is high phenolic and α-tocopherol content. They also observed that methanolic extracts had metal chelating attributes that were higher than BHA and α-tocopherol. Finally, *A. bisporus* demonstrates hypoglycemic effects mediated by polysaccharides that may help control glycemic level (Poucheret *et al.* 2006). It was mentioned in reviews by Sharma *et al.* (2014) and Alves *et al.* (2012) that antibacterial studies of *A. bisporus* were contradictory. They attributed conflicted

findings to the different extraction methods used and other differences between protocols.

Another factor, which may have contributed to variable results, is that *A. bisporus* circumscribes multiple varieties that differ in colour, size and other attributes. Therefore, it could be that different varieties were used in different studies that differ in activity.

***Armillaria* spp.** *Armillaria* spp. (common name 'Honey Fungus') have been previously studied for antibacterial properties. Several sesquiterpene esters showing antibacterial activity have been isolated from *Armillaria mellea sensu lato* (Donnelly *et al.* 1987; Donnelly *et al.* 1985). Compounds were tested against bacteria and had inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus* (Donnelly *et al.* 1985). Ethanolic extracts of the fruit-bodies inhibited the above two bacteria, as well as *B. cereus*, while ethanolic extracts of the mycelium inhibited *S. lutea*. In a separate study, ethanolic extracts of both fruit-bodies and mycelium inhibited several Gram-negative bacteria (Alves *et al.* 2012). Before 1990, *A. mellea* (*s. l.*) was thought to be a single species, however, based on mating interactions and DNA markers, *A. mellea* (*s. l.*) was resolved into several species (Anderson *et al.* 1987; Anderson and Ullrich, 1979; Korhonen, 1978). Therefore, it is uncertain which species had activity from the earlier studies. My study involves the evaluation of three different, well-delineated *Armillaria* species, *Armillaria mellea sensu stricto*, *Armillaria solidipes* and *Armillaria gallica*, that were identified through mating compatibility, mitochondrial and nuclear DNA RFLPs and DNA sequencing (Smith *et al.* 1990). This study should clarify the antibacterial attributes of these *Armillaria* species.

***Clitocybe nuda*.** Formerly known as *Lepista nuda* (common names include 'Blewit' and "Bluefoot"), this species is not well researched, although there are studies outlining its

antioxidant and antibacterial activity. Although lower than some other wild mushrooms, *C. nuda* produces a small amount of polyphenols and has antioxidant activity and reactive oxygen species (ROS) scavenging abilities (Elmastas *et al.* 2006). Alves *et al.*, (2012) summarizes that methanolic extracts show antibacterial activity against several bacterial organisms, *E. coli* included (Dulger *et al.* 2002), and was one of the most effective antibacterial mushrooms along with *Agaricus bisporus* (Ozen *et al.* 2011), *Lentinus edodes* (Hearst *et al.*, 2009), and *Ganoderma lucidum* (Quereschi *et al.* 2010). Biologically significant compounds have not yet been isolated from *C. nuda*, although some compounds have been isolated from other species of *Clitocybe*. The compounds nebularine and clitocypin were isolated from *Clitocybe nebularis*, which are potentially bactericidal and anticancer compounds, respectively (Poucheret *et al.*, 2006; Benedict and Brady, 1972; Brown and Weliky, 1953). The protein (N-terminal sequence SVQATVNGDKML) isolated from *Clitocybe sinopica* was antibacterial against *Agrobacterium* spp. and *Xanthomonas* spp. (Alves *et al.* 2012). Finally, *Clitocybe* sp. was reported as a potential treatment of metabolic disorders including Type II diabetes (Xu *et al.* 2009), and Shih *et al.* (2014) found that *C. nuda* exerted antidiabetic and hypolipidemic properties in streptozotocin (STZ) induced diabetic mice.

***Hypsizygus tessellatus*.** *Hypsizygus* spp., (commonly known as 'Beech Mushroom' or 'shimeji') are not well studied and not considered medicinal but are commonly found in the market and are edible. Of biological significance, *Hypsizygus marmoreus* (white beech mushroom) was recorded to exhibit antioxidant activity from extracted polysaccharides (Li *et al.*, 2012) and to have cholesterol lowering effects (Mori *et al.* 2008). When *H. marmoreus* was supplemented in animal diets, total serum cholesterol concentrations and atherosclerotic lesion formation

decreased rapidly in apolipoprotein E-deficient (apoE-) mice and was most effective in decreasing atherosclerotic lesions compared to *Grifola frondosa* and *Pleurotus eryngii*. To date, antibacterial studies have not been recorded for *Hypsizygus* spp.

***Ganoderma* spp.** The *Ganoderma* species evaluated in this study are *Ganoderma lucidum* (common names are 'Reishi' and 'Lingzhi') and *Ganoderma tsugae* (common name 'Hemlock Varnish'). Both are medicinal mushrooms, although not considered edible (Chang, 1996). Anti-inflammatory properties have been attributed to triterpenoids and steroids from both *G. lucidum* and *G. tsugae* (Ko *et al.* 2008). They are most often consumed as a hot water extracts (i.e. tea or tonic). *G. lucidum* is available in powders and dietary supplements (Wachtel-Galor *et al.*, 2011) and is regarded as "the herb of spiritual potency" and has a long history of medicinal use, dating back 2000 years in ancient Chinese scriptures and artworks. In TCM, it has been attributed with therapeutic properties such as tonifying effects, enhancing vital energy, strengthening cardiac function, increasing memory, and antiaging effects. In addition, according to the State Pharmacopoeia of the People's Republic of China (2000) the medicinal uses includes easing the mind, relieving cough and asthma, against dizziness, insomnia, palpitation, and shortness of breath. *G. lucidum* is considered valuable more for its pharmacological properties than nutritional value. Its recorded biological activities included antitumour, immunomodulatory, cardiovascular, respiratory, antihepatotoxic and pain relieving effects (Pouchert *et al.* 2006; Chang & Mshigeni, 2001; Ha *et al.* 2000). Known bioactive metabolites include terpenoids, steroids, phenols, nucleotides and their derivatives, glycoproteins, polysaccharides and glucans. The immunological protein LZ-8 and triterpenoids from *G. lucidum* induce the production of cytokines (ILs), Tumor Necrosis Factor Alpha (TNF α) and interferon

(IFN), and mobilize macrophages, NK cells, and lymphocytes B and T (Wachtel-Galor *et al.* 2011; Poucheret *et al.* 2006), holding potential application for immunomodulation and antitumour/anticancer treatments. *G. lucidum* extracts have antibacterial properties, including against *E. coli* (Sharma *et al.* 2014; Alves *et al.* 2012; Quereshi *et al.* 2010), with major active compounds including ganoderic acid, triterpenes and polysaccharides (Quereshi *et al.* 2010).

Not as thoroughly studied as its relative, *Ganoderma tsugae* has been investigated and found to have antioxidant activity (Tseng *et al.* 2008; Mau *et al.* 2005), anti-inflammatory properties (Ko *et al.* 2008), and potential anticancer properties (Yu *et al.* 2012). Methanolic and hot water extracts contain ascorbic acid and several phenolic compounds including tocopherol, and have overall high antioxidant activity (Mau *et al.* 2005). Extracts of fruit-bodies showed more antioxidant activity than extracts of mycelium or spent medium filtrates. *G. tsugae* produces polysaccharides which are thought to contribute to the antioxidant activity based on the study by Tseng *et al.* (2008). They performed antioxidant activity tests on the polysaccharide content of both hot water and methanolic extracts of *G. tsugae*, and proposed that the hot water and alkali extracted polysaccharides be developed as a dietary supplement or formulated into bread as a health-promoting functional food. Antibacterial properties have not been reported with this species.

***Grifola frondosa*.** Another medicinal, edible mushroom, *G. frondosa* (common name 'Maitake' and 'Hen-of-the-Woods') is traditionally used in folk medicine for its immune-boosting and overall health promoting properties (Poucheret *et al.* 2006). Ethanol extracts contain a polysaccharide composition of β -glucans with both a 1, 6 main chain having a greater degree of

1,3 branches, and a 1, 3 main chain having 1,6 branches (D-Fraction), and a unique β -glucan with more branching (Mayell, 2001). It is thought that these β -glucans play a role in immunomodulating effects, antitumor activity and glucose consumption by macrophages. It is thought that the higher branching β -glucan can reach more immune cells (Mayell, 2001; Adachi, 1990). *G. frondosa* also has an X-Fraction, containing a beta- 1,6 glucan having alpha-1,4 branches, and an MD-Fraction, having the same composition as the D-Fraction but in a more purified form. These β -glucan polysaccharides have been tested as antitumour agents by injection into carcinoma-transplanted mice. It was found that mice treated with the MD-Fraction experienced stronger inhibitory growth of the tumours (Mayell, 2001; Namba *et al*, 1998). Studies also showed that the MD-Fraction can induce T-cells and NK-cells, more so than the D-Fraction (Mayell, 2001), leading to studies into applications of the MD-Fraction in HIV and AIDS treatments (Poucheret *et al*. 2006; Kodama *et al*. 2002; Mayell, 2001). *G. frondosa* was also found to exhibit anti-inflammatory effects, with applications in atherosclerosis (Mori *et al*. 2008) as described above for *Hypsizygus tessellatus*. To date, antibacterial studies have not been recorded for *Grifola frondosa*.

***Boletus edulis*.** The edible mushroom *B. edulis* (common name 'Porcini', 'King Bolete') is known for its antioxidant activity associated with the presence of several compounds in the extracts, including flavonoids, anthocyanins, tocopherols and phenols (Vamanu and Nita, 2013; Tsai *et al*. 2006). Vamanu and Nita (2013) found that the composition of antioxidant compounds varied between different extractions performed and suggest that the ethanolic extract would be the most effective extraction for this mushroom. *B. edulis* is also known for its ability to thrive in soils that have been heavily contaminated with metals. Many studies have looked at the

mineral composition from different environmental habitats and found that the fruit-bodies contain a diverse mineral content and trace elements that may contribute to the nutritional value of this mushroom. These include the essential elements Cu, Fe, K, Mg, Mn, Na and Zn and several non-essential elements including Al, Ba, Ca, Cd, Hg and Sr (Zhang *et al.* 2010). In terms of antimicrobial activity, *B. edulis* showed antibacterial effects on several bacteria, including *E. coli* and *Staphylococcus aureus* (MIC=5µg /ml), at a lower concentration than ampicillin (MIC=6.25µg /ml) (Alves *et al.* 2012; Barros *et al.* 2008). Three peptides isolated from *Boletus* spp., peptaibol boletusin, peptaibol chrysospermin 3, and peptaibol chrysospermin 5, are all known for the opening of ion transport pores (Lee *et al.* 1999) and were found to be the active compounds against the bacteria (Alves *et al.*, 2012).

Antimicrobial Activity Detection

Extraction methods, growth conditions and other environmental cues, in addition to tissue type (e.g. mycelium vs fruit-body), may all influence antimicrobial production processes. A review by Alves *et al.* (2012) suggests that different extracts of the same species have significant differences in antibacterial activity. Depending on the species, activity is often seen in the fruit-bodies or the mycelium. In previous studies reviewed by Alves *et al.* (2012), several fungi were tested via different extraction methods and the most effective extraction methods differed depending on the species.

A study by Williams *et al.* (2008) described methods to induce secondary metabolite activity in fungi by upregulating the expression of silent genes with epigenetic modifiers and by introducing a bacterial antagonist into the environment. This was presented as a rational approach to activate the expression of silent genes, opening up new biosynthetic pathways that

would result in the fungi producing compounds they would normally not produce. Methylation of the genome is as an epigenetic phenomenon known to silence the expression of genes. Loss of methylation, therefore, leads to the opposite effect- activated gene expression. 5-azacytidine is a methyltransferase inhibitor that can act as a small-molecule epigenetic modifier. It is toxic at high concentrations but at a lower dose this chemical reduces the incidence of methyl groups on DNA, and thus reduces gene silencing. This ultimately results in hypomethylation of the genome during successive DNA replication cycles (Martens, 2010; Williams *et al.* 2008).

Treatment with 5-azacytidine in the study by Williams *et al.* (2008) resulted in the production of several oxylipins from *Cladosporium cladosporioides*. Oxylipins are of great interest since these metabolites are known for their role in intra- and inter-species cell signaling molecules. 5-azacytidine also significantly changed the metabolic profile of *Diatrype disciformis* causing it to produce two new polyketides, lunalides A and B. Similarly, Williams *et al.* (2008) found these same two polyketides were produced by *D. disciformis* following addition of spent *Escherichia coli* filtrates. These polyketides were otherwise not produced in axenic cultures. In this case, the spent *E. coli* filtrates may act as an environmental cue, causing the fungus to release metabolic/chemical defenses.

Nutrient stress may also cause expression of novel secondary metabolites. A study on production of the antibiotic fumagillin produced by *Aspergillus fumigatus* showed that limiting the growth medium to 1% glucose resulted in highest yields of fumagillin. The authors also found a reduction in yield of fumagillin in medium with 3% glucose in which there was maximum production of fungal biomass (Barborakova *et al.* 2012). Limited or non-preferred nitrogen supplies can also stress fungi and result in increased yields of secondary metabolites.

In *Fusarium fujikuroi*, for example, the secondary metabolites gibberellin, bikaverin and carotenoids were absent in a medium with abundant ammonium nitrate but were produced under reduced nitrogen environments (Rodriguez-Ortiz et al., 2009). Similarly, in *Fusarium graminearum*, DON production is associated with non-preferred nitrogen sources of agmatine and putrescine while preferred nitrogen sources of glutamine, asparagine and ammonia repress DON production genes (Walkowiak and Subramaniam, 2014; Gardiner et al. 2009a).

Rational & Objectives

My study evaluated the antimicrobial activity of fourteen edible/medicinal basidiomycete fungi, of the phylum Basidiomycota, subkingdom Dikarya. Dikarya also consist of another phylum, Ascomycota. Both phyla are often referred to as higher fungi but differ in reproductive characteristics. In basidiomycetes, spores, called basidiospores, are produced externally on specialized cells, the basidia, and ascomycete spores, ascospores, are produced internally in a sac called the ascus. Basidiomycete 'antagonists' were tested *in-vivo* against a bacterial or fungal test organisms, *E. coli* and *S. cerevisiae*, respectively, in which antimicrobial activity was recognized by the formation of inhibition zones in a lawn of test organism cells. Ethanol extracts of fungal cultures were also tested against *E. coli* (wild type and two fluoroquinolone-resistant mutants), and against *S. cerevisiae*. Attempts were made to 'induce' cultures of the fungal antagonists to produce inhibitory secondary metabolites by addition of the epigenetic modifier 5-azacytidine, addition of spent bacterial antagonist and by using nutrient-limited growth medium. The treatments can be used to discover antibacterial candidates- whether treatments cause production of new compounds or cause an increase in production of existing compounds. Again, the use of edible/medicinal fungi was rationalized for

study as they are regarded as safe for use and consumption and have biological indications based on traditional uses. While traditional uses involve administering the fruit bodies, my study will be done using fungal mycelia, as these are more appropriate for commercial production of antimicrobials, and can be easily exposed to different induction treatments. Extracts of both the mycelial portions and the culture filtrates were tested for antimicrobial activities.

SECTION II: Materials and Methods

Growth of mycelia

Basidiomycete cultures were obtained from fruit-bodies collected from commercial sources or from the field, or from culture collections (Table 1). For obtaining axenic cultures from commercial and field material, fungal tissue was excised from the inside of caps of the fruit-bodies with sterile forceps and transferred to potato dextrose agar (PDA) plates. Mycelia were allowed to grow for 2-4 weeks at room temperature after which 5 mycelial plugs (5 mm diameter) were transferred to 50 ml of potato dextrose broth (PDB) and allowed to grow at

Table 1. Scientific Latin and common names of specimens and their source.

Species name	Common name	Source
<i>Lentinula edodes</i> [(Berk). Pegler]	Shiitake	Collection LTSH. L. Tabaja; Carleton U. Commercial source.
<i>Pleurotus eryngii</i> [(DC). Quel.]	King oyster	Collection LTKO. L. Tabaja; Carleton U. Commercial source.
<i>Pleurotus ostreatus</i> [(Jacq.) P. Kumm.]	Oyster	Collection LTOY. L. Tabaja; Carleton U. Commercial source.
<i>Armillaria solidipes</i> [(Peck) Baroni]	Honey Fungus	430-7; M.L. Smith; Carleton U. Culture collection.
<i>Armillaria gallica</i> [(Marxm). & Romagn.]	"Humungous Fungus"	445-3; M.L. Smith; Carleton U. Culture collection.
<i>Armillaria mellea</i> [(Vahl). P. Kumm.]	Honey Fungus	422-3; M.L. Smith; Carleton U. Culture collection.
<i>Clitocybe nuda</i> [(Bull.) H.E. Bigelow & A. H. Sm.]	Bluefoot; Blewit	Collection LTBF. L. Tabaja; Carleton U. Commercial source.
<i>Agaricus bisporus</i> [(J. E. Lange) Imbach]	Portobello	Collection LTPB. L. Tabaja; Carleton U. Commercial source.
<i>Ganoderma tsugae</i> (Murr.)	Reishi, Hemlock varnish	J. Mack, M.L. Smith; Carleton U. Field collection.
<i>Hypsizygus tessellatus</i> [(Peck.) Bigel]	Brown Beech shimeji; Bunashimeji	Collection LTBS. L. Tabaja; Carleton U. Commercial source.
<i>Laetiporus sulphureus</i> [(Bull.) Murrill.]	Sulphur shelf	J. Mack, M.L. Smith; Carleton U. Field collection.
<i>Ganoderma lucidum</i> [(W. Curt.: Fr.) Karst.]	Reishi	DAOM 10222; AAFC. Culture collection.
<i>Grifola frondosa</i> [(Dicks.) Fr.] S.F. Gray]	Maitake	DAOM 221498; AAFC. Culture collection.
<i>Boletus edulis</i> [(Bull.) Fr.]	Porcini, King Bolete	Collection #2; J. Tanney; Carleton U. Field collection.

room temperature for 2-4 weeks. Once an adequate biomass was obtained, a stock culture was made by blending the mycelium using a sterile blending cup and a Waring Commercial Blender for 30 seconds, 3 times, with a 30 second pause between pulses. Aliquots of blended mycelium were mixed with sterile glycerol (15% final concentration) and stored as frozen stocks at -80 °C. In addition, working stocks were stored on PDA plates at 4°C.

Induction Treatments

Nutrient-limited medium induction

One ml of stock inoculum was transferred to a 250 ml Erlenmeyer flask containing 50 ml of fresh PDB and allowed to grow for 2-4 weeks. After an adequate amount of growth, the mycelium was pelleted at 3050 x g for 8 minutes, rinsed with sterile distilled water, pelleted again by centrifugation, and the supernatant was discarded. The mycelium was then transferred to a 250 ml Erlenmeyer flask containing 50 ml nutrient limited medium [0.1% (NH₄)₂HPO₄, 0.3% KH₂PO₄, 0.02% MgSO₄•7H₂O, 0.5% NaCl, 0.1% Sucrose, 1% Glycerol]. The cultures were allowed 1 additional week of growth and then vacuum filtered through Whatman #1 paper in a Buchner funnel. The mycelium and medium filtrate for each culture were separately retained at -20°C until freeze-drying.

Killed *Escherichia coli* culture induction

E. coli was prepared by inoculating a single colony of strain MG1655 obtained from the laboratory of Dr. Wong (Carleton University) into 6 ml of LB broth and incubated overnight at 37 °C with shaking 100 rpm. One ml of the bacterial culture was then transferred into 200 ml LB broth and incubated at 37 °C with shaking at 100 rpm for 48 hours at which time the culture had reached stationary phase. The *E. coli* cell cultures were then mechanically disrupted using

0.1 mm Biospec glass beads and a Biospec Bead Beater (Biospec Products, Bartlesville, Oklahoma) for 3 minutes, at high speed. Chloroform (3 – 4 drops) were then added to the culture and the mixture was heated in a 70°C water bath for 20 minutes. The chloroform and hot water bath steps were repeated until no live cells were detected based on viability counts following plating an aliquot of the mixture onto LB agar. To prepare the fungal culture, 1 ml of blended fungal inoculum was added to 50 ml fresh PDB and incubated for 2-4 weeks at room temperature, as described above, prior to the 'induction treatment'. On days 1 and 4 of the 'induction treatment', *E. coli* dead cell mixture was added to the liquid culture of fungal mycelium at a ratio of 1:2 both times (dead *E. coli* culture: fungal culture). On day 7 after the first induction treatment, the mycelium and filtrate were separately harvested as described above. The mycelium and medium filtrate were each retained at -20°C until freeze-drying.

5-azacytidine induction

One ml of blended fungal inoculum was transferred to 50 ml fresh PDB, as described above, and after 2-4 weeks of growth, 0.1 μ M 5-azacytidine (5-AC) was added to the culture in 1:2 ratio both times (5-AC: fungal culture). Addition of the same volume of 5-AC was repeated after 4 additional days, and on day 7 after the initial induction treatment the mycelium and medium were separated as described above and stored separately at -20°C until freeze-drying.

A 4th, 'un-induced' fungal culture was grown as a control, where 1 ml fungal inoculum was grown in 50 ml LB and filtered at the same time points as for the induced treatments. As in induction treatments, medium and mycelial fractions were retained separately at -20 C until freeze-drying for extractions.

Crude Extractions

Frozen medium and mycelium samples obtained from filtration were freeze-dried (Labconco Freezone¹² Kansas City, Missouri) at -50°C, 0.05 mbar until dry (4-5 days). The lyophilized medium portions were re-suspended in 15% ethanol and stored at -20°C until use. Mycelial samples were crushed into a fine powder using liquid nitrogen and a mortar and pestle and extracted with 80% ethanol at a 1:10 ratio (mycelium: solvent, w/v) by shaking at 100 rpm for 48 hours at room temperature in an Innova 40 Shaker Incubator (Eppendorf, New Brunswick). Extracts were then filtered through Whatman #1 paper using a Buchner funnel. The mycelial residue was discarded and the remaining ethanol in the supernatant was evaporated by rotary evaporator (Buchi, New Castle, Delaware) and then stored at -20°C after which they were freeze-dried again for 24 hours. The lyophilized extracts were dissolved in 15% ethanol by sonication (Branson 5510 sonicator, Richmond, Virginia), and stored at -20 °C until further use.

Top Agar Susceptibility Bioassay

As a preliminary study, top agar assays were performed with each basidiomycete fungus (antagonist) against *E. coli* (MG1655) and *Saccharomyces cerevisiae* (S288C) (test organisms) to test for antibacterial and antifungal activity, respectively. This involved growing the antagonist on PDA plates for 3-5 days until a colony diameter of approximately 5 mm was reached. A top agar (LB + 0.8% agar for *E. coli*, PD + 0.8% agar for yeast) was prepared and cooled to 55°C before addition of $\sim 10^5$ CFU/ml *E. coli* or $\sim 10^4$ CFU/ml *Saccharomyces cerevisiae*. The medium was mixed briefly and poured over the surface of the agar plate containing the antagonist colony. The plates were incubated for 1 day at 37°C (*E. coli*) and 1 days at 30 °C (yeast) and diameters of any observed zones of inhibition were recorded.

Fungal species with activity against *E. coli* were then tested against the *E. coli marR* and *gyrA* mutants in the same manner.

Confirmation of Ciprofloxacin Resistance in E. coli mutants

To confirm the resistance of the *marR* (R77H) and *gyrA* (S83L) mutants, and sensitivity of a wild-type (MG1655) *E. coli* strains, $\sim 10^5$ CFU/ml of each *E. coli* strain was inoculated separately onto LB agar medium containing 25 ng/mL ciprofloxacin (cipr-LBA) and, at the same time, onto LB agar medium. The plates were incubated at 37 °C for 24 hrs. Growth of *marR* and *gyrA* mutants and no growth of MG1655 on cipr-LBA compared to growth by all strains on LBA medium would confirm the ciprofloxacin-resistance/sensitivity of *E. coli* strains.

Minimum Inhibitory Concentration Assays

Minimum inhibitory concentration (MIC) assays were performed with mycelium and culture medium extracts against *E. coli* and *S. cerevisiae* using a Viaflow Assist automated liquid handler (Integra, Hudson, New Hampshire). Three strains of *E. coli* were tested (final concentration of $\sim 10^5$ CFU/well in a final volume 200 μ L/well), MG1655 (wild-type), R77H (*marR* mutation), and S83L (*gyrA* mutation). The *S. cerevisiae* strain used was S288C (final concentration of $\sim 10^5$ CFU/well, final volume 200 μ L/well). A standard MIC protocol was used (Wiegand *et al.* (2008) with a 100 μ L crude extract starting volume sequentially diluted 1:1 across the plate. The *E. coli* inoculum contained 20 mM citric acid (3.84% citric acid, pH 5.0) to permeabilize the bacterial cell membrane. Previous studies have demonstrated that chelators including EDTA, lactic acid and citric acid can be used to disrupt the outer membrane of Gram-negative bacteria, permeabilizing the cells, and increasing their sensitivity to antimicrobial agents (Galvan, 2016; Alakomi *et al.*, 2000). MIC plate layout outlines are provided in Appendix I. As controls,

hygromycin B and ampicillin were used as positive antibiotic controls for *S. cerevisiae* and *E. coli*, respectively. Three carrier controls were also used: LN-medium, LB-medium containing *E. coli* dead cell mixture (prepared as above) and LB-medium containing 0.1 μ M 5-AC. Controls were prepared using the same procedures used for preparing the mycelium and culture medium extracts of antagonists, and were dissolved at 50 mg/mL in 15% ethanol. MICs with *E. coli* were incubated for 20 hrs at 37 °C, and with *S. cerevisiae* for 48 hrs at 30 °C, after which absorbance readings were made at 600 nm using a BioTek Cytation 5 image reader and Gen5 software. Extract concentrations giving 100%, 90% and 50% growth inhibition were recorded as MIC₁₀₀, MIC₉₀ and MIC₅₀, respectively.

Monitoring for Biocidal and Biostatic Activity

Following MIC assays, extracts having full microbial inhibition (MIC₁₀₀ values) were tested for bactericidal (or fungicidal) or bacteriostatic (or fungistatic) properties. This was done by inoculating 50 μ L of medium from the MIC₁₀₀ well onto an agar plate (LB for *E. coli* and YPD for *S. cerevisiae*). If growth was evident within 3 days at 37 °C (*E. coli*) or 30 °C (*S. cerevisiae*), the affects were recorded as being bacteriostatic or fungistatic, respectively. If no growth was evident after 3 days, the affects were recorded as bactericidal or fungicidal, respectively. In addition to plating MIC₁₀₀ wells, I also inoculated each of the three adjacent test wells that had incrementally lower crude extract concentrations, and where some microbial growth was observed. Following incubation, CFU counts were used to confirm biocidal activity (an increase in CFU count occurred with decreased crude extract concentration), and biostatic activity (a constant CFU count occurred with decreased crude extract concentration). A similar, second method employed inoculating 100 μ L of the well-

contents, from the test well with no microbial growth, into 2 mL of fresh medium (LB for *E. coli* and YPD for *S. cerevisiae*) followed by 24 hrs incubation at 37 °C for *E. coli* or 48 h incubation at 30 °C for *S. cerevisiae*. If growth was observed within 3 days, the affects were recorded as

bacteriostatic/fungistatic and if no growth occurred the affects were recorded as bactericidal/fungicidal.

Spawn-on-Logs

Since the fruit bodies of the mushrooms I looked at in this study can be proposed as a food therapy or functional food, as a side project, I chose four fungi to inoculate in logs to produce fruit bodies. I chose *Lentinula edodes*, *Laetiporus sulphureus*, *Ganoderma lucidum* and *Pleurotus ostreatus* to grow in logs due to their natural occurrence on deciduous trees. Mycelia were first grown in jars

Figure 1. Procedure of log inoculations. Top two left pictures show the inoculated spawn (sawdust/wheat/mycelia mixture). Hardwood logs were drilled along the length of the trunks (top right), the middle picture shows the inoculation of spawn in the holes. Bottom picture shows the final products after the holes' have been sealed with wax.

of sterilized sawdust and wheat before being used to inoculate logs. Sawdust of sugar maple was first soaked in water for 24-48hrs, drained, and mixed thoroughly with wheat kernels (4 parts sawdust: 1 part wheat). Clean wide-mouth jars were 3/4 filled with the mixture, covered loosely with the jar lid and aluminum foil, and autoclaved for 40 minutes. Once cooled, mycelial plugs from 2-3 PDA plates of each basidiomycete fungus were added separately to the jars using sterile technique. The jar lids and aluminum foil were loosely reattached to allow for gas exchange and the jars were placed at room temperature under ambient light for 3-4 weeks to allow for growth. The jars were occasionally shaken to distribute mycelia within the sawdust/wheat mixture. Once the sawdust-wheat substrate was completely colonized by fungal mycelium (about 4 – 8 weeks), the spawn was packed into ~2 cm diameter x 6 cm deep holes bored in *Fraxinus pennsylvanica* (green ash) logs. The holes were then sealed with warm paraffin wax and logs were stacked and left in the Carleton Biology Research and Teaching Garden (Fig. 1).

SECTION III: Results

Characteristics of Basidiomycete (Antagonist) Fungi Used in this Study

Lentinula edodes. Native of Japan, *L. edodes* is a saprophytic basidiomycete that colonizes hardwood trees and logs. Its fruiting body has a tough, fleshy brown to dark brown cap with white veil remnants, a central stalk, white gills and a white to buff spore print. In liquid culture, the mycelium is white to buff, and grows very slowly- about four weeks to colonize at with an adequate biomass. Even after several months (4-5 months), the mycelia do not colonize the full volume of the flask. Optimal biomass was obtained after about one month in potato-dextrose broth (PDB) at room temperature, after which the mycelium becomes fragile and became dull in colour, likely due to senescence. On potato dextrose agar (PDA), the mycelium grows white in colour and turns brown when mature. The mycelium grows slowly in a radial pattern with a regular colony margin on PDA, taking about one month to reach the edge of the plate at room temperature (Fig. 2). Fruit-body primordial can form at later stages of growth on PDA.

Pleurotus spp. Species of this basidiomycete genus grow on herbaceous and woody substrates. The fruit-bodies are medium to large in size, shelf- or fan-like in shape, with a lateral stalk.

Pleurotus ostreatus has soft fleshy white to grayish-brown fruit-bodies that often grow on hardwood logs and stumps in overlapping groups. The fruit-bodies have white gills and a white to buff to pale lilac spore print. Native to Europe and Asia, *Pleurotus eryngii* grows on decaying herbaceous plants, unlike most of its *Pleurotus* spp. relatives. It has a tough fruit-body, with a small, brown cap and a white, thick, lateral stalk. It has brown gills and a white spore print. The mycelia of both *P. ostreatus* and *P. eryngii* grow relatively rapidly in PDB as a white and fluffy mycelium. Within a month, the biomass can take 50 ml of PDB medium, and even grow up the

sides of the flask, without appearing stressed (i.e. no colour or texture change). On PDA, the mycelium grows radially in a fibrous manner, with a smooth colony margin. The fibrous mycelium grows rapidly, reaching the edge of the plate within two weeks and does not appear stressed with time (i.e. no colour or texture change), although pale yellow spots can be seen in *P. ostreatus* throughout the growth period and fruit-body primordia can form at later stages of growth (Fig. 3).

***Laetiporus sulphureus*.** A bracket/shelf fungus, this saprophytic basidiomycete grows in an overlapping, shelf-like clusters on dead or live trees, on both hardwoods and conifers. The fruit-bodies are yellow to orange and pliable when immature but can become leathery in texture with age. The caps are flat and can grow up to ~70 cm broad and ~4 cm thick in fan-like shape, the stalk can be absent or present as a narrowed base, emerging from the host tree. The fruit-bodies have a white spore print. In liquid culture (PDB), the mycelium is hydrophobic and will usually float on the surface of the medium and grow as a fluffy, bright orange mat. Within a month, the mycelium can grow to occupy 50 ml of PDB at room temperature, although it does not take up the entire volume of the media. The colony colour and texture remain stable with age up to at least one month. In PDA, the mycelium grows radially as a bright orange, smooth mycelium with prominent rings of pigment that resembles the annual rings of a tree-trunk. With time, the mycelium will form a soft powdery growth, appearing very fluffy but very fragile, as it disintegrates when touched. It can colonize the entire plate of PDA medium within two to three weeks at room temperature (Fig. 4).

***Agaricus bisporus*.** Fruit-bodies of this species are commonly found scattered or as dense groups in rich pasture soils or compost, and rarely in forest or lawns. They have rounded caps

that are usually white but can become brown with age depending on the strain, and can grow to a very large size depending on the strain. They have brown gills at maturity and a dark brown spore print. The fruit-body evaluated in this study was the “Portobello”, which have large caps of ~15 cm diameter. In liquid PDB medium, the mycelium grows very slowly, appears fragile and is a light brown colour. After about one month growth at room temperature, the mycelium will grow out only about 5 mm from the inoculated plugs. In PDA, the mycelium grows light brown, in an irregular radial pattern, and grow very slowly- it may take several months for mycelia to reach the edges of the plate when incubated at room temperature (Fig. 5).

***Armillaria* spp.** This genus consists of terrestrial forest-dwelling mushrooms that are facultative pathogens of trees. The fruit-bodies are a honey-brown to dark brown, with a convex cap and a fragile membranous veil. The base of the stalks often appear swollen (bulbous) and the fruit-bodies often grow in clusters attached at the base. The gills are white to yellow, with a white spore print. The three *Armillaria* species used in my studies are *A. mellea*, *A. solidipes* and *A. gallica*. In PDB, the mycelium grows in a thick bulky mass; *A. gallica* will readily grow branch-like rhizomorphs appearing brown in colour and the other two species will sometimes produce rhizomorphs as well. All species grow relatively rapidly, taking up the 50 ml volume PDB medium within a month at room temperature with a white to brown mycelium, and will turn the medium blood-red to red-brown with time. In PDA, the mycelia of all three species grow in an irregular radial pattern. *A. gallica* will readily grow rhizomorphs through the agar, *A. solidipes* will sometimes produce rhizomorphs, and *A. mellea* produces rhizomorphs infrequently. On PDA, the mycelia of *A. mellea* and *A. gallica* appear brown in colour and *A. solidipes* mycelium appears white to dull beige in colour. When growth starts, the agar

surrounding the inoculum plug turns a faded red colour and with time, all three species turn the agar on the entire plate a deep red to brown colour. The three species begin to “bleed” red droplets on the surface of the mycelium. Growth is relatively slow; after about one month of growth at room temperature on PDA, the diameter of the mycelium covers $\frac{3}{4}$ of the plate and doesn’t usually grow to the plate edge (Fig. 6).

***Clitocybe nuda*.** This mushroom is found in the field on the base of living trees, namely oaks, pine or cypress but can also be found on any kind of decomposing matter, including compost, shredded newspapers and organic debris. *C. nuda* has a relatively short, thick stalk and a large smooth, usually umbonate-shaped cap. With age the stem and cap may turn pale blue to deep purple, with blue or purple or pale pink gills, although with age the fruit-bodies can also become a dull brown colour. Spores are pale pink and spore print is pink. In PDB, the mycelium of this fungus grows extremely slowly. The mycelium may take up to a month to begin to visibly grow out from the plug. After one month of growth at room temperature in PDB, the result may be a ~5 mm mycelial growth around the inoculation plug. The mycelium appear appressed and fragile and after several months the mycelium can turn a pale blue or purple colour. In PDA, the mycelium grows faster compared to in liquid PDB, however it is still quite slow-growing. At room temperature on PDA, it may take two months for the radial growth to reach the edge of the plate. The mycelium has an irregular radial pattern of growth on PDA, with centre of the colony growing upwards to touch the lid of the plate, with the rest of the mycelium being flat (appressed) as it grows toward the edge of the plate. The mycelium will grow as a dull blue colour and this colour will intensify with time until, with age, the colony will begin to dullen to appear a beige-blue colour (Fig. 7).

***Hypsizygus tessellatus*.** The fruit-bodies of this basidiomycete are tough, with a long skinny white stalk and small umbrella-shaped brown cap with white gills. The species is native to Europe and Asia and mostly found on beech trees as clusters of mushrooms. In both PDB and PDA, the mycelium is white and fluffy. In liquid, the mycelium appears to float on the surface and produce white, cottony tufts growing upwards. In agar plates, the mycelium begins to grow in a fibrous, irregular manner that turns to white cottony growth form that can reach the edge of the plate in a few weeks at room temperature on PDA. After a few more weeks of growth, the mycelium becomes a thick, leathery-tough growth (Fig. 8).

***Ganoderma* spp.** The polypore mushrooms in this genus are commonly known as “conks” for their tough, woody texture and presence of a hard surface crust. Fruit-bodies can be hoof or fan-shaped and can grow quite large, with some specimens achieving a diameter of ~75 cm. *Ganoderma lucidum* can grow up to ~35 cm in diameter, ~8 cm thick with a shelf-like or hoof-shaped brown cap. It has a ‘varnished’ surface crust, often grooved with smooth edges. The cap is dark-brown or reddish-brown, often with a white or yellow margin. The stalk is often absent, but when present is attached laterally, dark brown or red in colour, appearing varnished as the cap. The underside of the cap has white or yellow, minute pores and a brown spore print is produced. *G. lucidum* fruit-bodies are normally found at the base, roots or stumps of living hardwood trees, and rarely on conifers. In culture, *G. lucidum* grows as a white mycelium that starts out fluffy but eventually becomes a toughened, leathery mass, floating on the surface as a thick mycelial clump. On PDA, *G. lucidum* mycelium is white and grows in an irregular, radial pattern, appearing flattened/appressed to the agar surface. *G. lucidum* grows relatively fast on PDA, reaching the edge of the plate in about two weeks at room temperature. *Ganoderma*

tsugae is very similar in fruit-body shape and, also, has the varnished surface appearance. This mushroom is only found on conifers, usually hemlock, and has white flesh, although aged segments of *G. tsugae* appear brown. In PDB, *G. tsugae* initially has a similar appearance to *G. lucidum* on PDB, but after time, segments turn light brown to greenish-yellow with white margins. On PDA, *G. tsugae* mycelium is similar to *G. lucidum* in having a flattened, irregular, radial pattern and at a similar growth rate, but with age, segments of the mycelium begin to appear light-brown to greenish-yellow. Mycelia for both species are tough and leathery with age (Fig. 9).

***Grifola frondosa*.** Another polypore, this mushroom is usually found growing at the base of oak trees or stumps. The fruit-bodies grow in clusters, usually seen as numerous caps overlapping one another, and emerging from a common fleshy, branched base. The caps appear thin or flattened, fan or tongue-shaped with a dry smooth or rough surface, and with a wavy margin. The fruit-bodies appear brown-grey in colour with stalks that are white or pale grey, smooth and fleshy but tough, often attached to the sides of the caps. The pores on the underside of the cap are white or yellow and shallow and produce a spore print that is white. The mycelium in PDB appears fluffy and white and eventually forms a thick biomass of fluffy mycelium floating on the surface of the medium with a fibrous textured growth within the medium. On PDA, the mycelium grows in a fibrous manner and eventually forms a thick and firm texturized mass that is initially white in colour, turning a dull yellow in some areas with time. It takes only 2-3 weeks for the mycelium to grow to the edge of the plate when incubated at room temperature (Fig. 10).

Boletus edulis. The fruit-bodies of this mushroom include a thick white stalk, and a convex red to brown cap. The cap margin can be white or yellow, and on the cap underside the pores are white, becoming yellow to olive-green, with a spore print that is olive brown. Flesh of the mushroom is thick and firm. In PDA, the fungus begins to grow radially as a white fluffy-textured mycelium, at a very slow rate, reaching about 5 mm diameter after 3 - 4 weeks. The mycelium will continue to grow from the origin to form a thick, white textured mass, resulting in what appear to be tiny caps, or spherical masses – likely fruit-body primordia. In PDB, the mycelium grows as a fluffy mass at a slow rate of about 5 mm/month. The mycelium begins as a white to dull brown colour but turns brown with age. After several (4-5) months of growth in PDB, plugs of inoculum are surrounded with a thick and firm mycelium but do not, however, take up the full volume of the medium (Fig. 11).

Observations of mycelia in post-induction treatments were generally the same among the different species. After the addition of both EC and 5-AC treatments, the mycelia grew slower (or halted growth) and often became less colourful in comparison to un-induced cultures. LN treatment resulted in a lightening in colour of the mycelia, which may be partly due to the clear colour of the LN medium. In addition, LN treatments resulted in reduced growth rates of the mycelia and apparent fragmentation of mycelia.

Figure 2. *Lentinula edodes* fruit-bodies (lower left) and mycelium on PDA (top left) and in PDB (right). Note the formation of a fruit-body primordium on PDA plate after four months of growth at room temperature (arrow).

Figure 4. *Laetiporus sulphureus* mycelial cultures on PDA (top left) and in PDB (right), and fruit-body on hardwood tree (bottom left). Both mycelial cultures aged one month at time of photo.

Figure 3a. *Pleurotus ostreatus* inoculated in PDA (top left = reverse side of plate, top right = top side of plate), aged eight months at time of photo. Fruit bodies depicted on ash (bottom right, photo by J. Mack) and from commercial source (bottom right).

Figure 3b. *Pleurotus eryngii* mycelial cultures on PDA, aged seven months at time of photo, and fruit body from commercial source.

Figure 5. *Agaricus bisporus* fruit-bodies (left) and mycelial cultures on PDA (top right = reverse side, bottom right = upper side).

Figure 6. *Armillaria solidipes* in mycelial culture on PDA (top left) and fruit-bodies (bottom right M.L. Smith). *Armillaria mellea sensu stricto* mycelium on PDA (bottom left) after two months' growth at room temperature. Note the brown colour in agar medium as the culture ages, as well as red droplets of fluid at the centre of the colony. *Armillaria gallica* rhizomorphs and mycelium on PDA after two months' growth at room temperature (top middle). Note the red-orange droplets at centre of mycelium. Bottom middle depicts rhizomorphs of *Armillaria solidipes* growing in liquid medium. Top right corner depicts *Armillaria solidipes* in the field. Field pictures as well as liquid culture taken by M.L. Smith.

Figure 7. *Clitocybe nuda* fruit-bodies and cultures. Top right is mycelial culture in PDB, after 3 months growth at room temperature at time of photo. Bottom right depicts two mycelial plugs inoculated on PDB, cultured for two months at room temperature at time of photo. Fruit-body pictures taken in the field by Jonathon Mack.

Figure 8. *Hypsizygos tessellatus* fruit-bodies from commercial source and cultures on PDA (upper side of plate culture in middle panel, reverse side in right panel).

Figure 9. *Ganoderma* spp. Top left corner shows mycelia of *Ganoderma lucidum* growing on PDA. Bottom left corner depicts mycelia of *Ganoderma tsugae*. Both cultures are aged one month at time of photo. Fruit-body pictures include *Ganoderma tsugae* growing on conifer wood (middle panel, immature fruit-body and top right), and *Ganoderma* sp. at bottom right corner. Fruit-body pictures taken by Jonathon Mack.

Figure 10. *Grifola frondosa* mycelium on PDA. Top panel is reverse side of plate, bottom is top side of plate.

Figure 11. *Boletus edulis* inoculated in PDB (top left) and PDA (bottom left), six months at time of photos. Note colour change with age in the liquid medium. Field picture of *Boletus chippewaensis* (the American equivalent of the European only *Boletus edulis*) on the right taken by J. Mack.

Confirmation of Resistance/Sensitivity to Ciprofloxacin by *E. coli* strains

Upon inoculation of *E. coli* strains on LB containing 25 µg/ml ciprofloxacin and incubation at 37°C for 24 hours, growth of both *cipR*-mutants, *marR* (R77H) and *gyrA* (S83L), was observed while no growth of the wild-type MG1655 *E. coli* was observed (Fig. 12). All strains grew on control medium that contained no ciprofloxacin. This confirms the resistance/sensitivity status

Figure 12. Agar inoculations of *cipR* mutants *gyrA* S83L (left), *marR* R77H (middle) and wtMG1655 (right). The agar contains LB growth medium for *E. coli* and 25ng/mL of ciprofloxacin to observe resistance in the mutants.

to ciprofloxacin for these *E. coli* strains.

Top Agar Bioassays

Figure 13 depicts an example of top agar bioassays performed with *Lentinula edodes*. In this figure, we see a clear halo around each mycelial plug of *L. edodes* (antagonist) wherein the growth of

test organisms was inhibited. Mycelia of *L. edodes* were able to inhibit growth of all three *E. coli* strains (the two *cipR*-mutants as well as the wild type) and the yeast *S. cerevisiae*. Diameters of the halos were measured and are presented in Table 2 for all 9 basidiomycetes with (+) inhibitory activity in this assay. Grades were also assigned based on qualitative and quantitative observations; qualitative observations- no inhibition was graded 0, inhibition just around the diameter of the mycelial plug received a grade of 1 and diameter of inhibition that extended beyond the diameter of the mycelial plug received a grade of 2. Of the fourteen basidiomycete (antagonists) species tested, *Lentinula edodes*, *Agaricus bisporus*, *Armillaria mellea* and

Clitocybe nuda demonstrated both antibacterial activity against all three *E. coli* strains tested and antifungal activity against *S. cerevisiae*. *Armillaria solidipes* demonstrated antifungal activity against *S. cerevisiae* and antibacterial activity against wtMG1655 and *marR* *E. coli*, but not the *gyrA* mutant *E. coli*. *Laetiporus sulphureus* demonstrated only antifungal activity against *S. cerevisiae*, and *Ganoderma tsugae* demonstrated antibacterial activity against the wtMG1655 *E. coli* alone. Both *Ganoderma lucidum* and *Grifola frondosa* demonstrated antibacterial activity against all three *E. coli* strains but no antifungal activity against *S. cerevisiae*. *Armillaria gallica*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Hypsizygus tessellatus* and *Boletus edulis* did not demonstrate antifungal or antibacterial activity and are, therefore, not included in Table 2.

Figure 13. Bioassays of *Lentinula edodes* (antagonist) against bacterial and fungal test organisms. The blue lines in the left panels and bottom right panel indicate inhibition zone diameter measurements. Top left panel shows *S. cerevisiae* test organisms. *E. coli* test organisms are as follows: Wild-type *E. coli* (MG1655, top right), *marR* mutant (R77H, bottom left) and *gyrA* (S83L, bottom right).

Table 2. Inhibition zone diameters (cm) from top agar bioassays with four microbe test organisms [*S. cerevisiae* (S288C), *E. coli* wild-type (MG1655), *marR* (R77H), and *gyrA* (S83L)], and 9 fungal antagonists. *A. gallica*, *P. ostreatus*, *P. eryngii*, *H. tessellatus* and *B. edulis* produced no inhibition zones in any of the bioassays and, therefore, are not included in this table. Mean inhibition zone diameters are given in cm (n=8) along with standard deviation (std dev) and standard error (std err) values. 'Grades' are also presented where 2=clear zone of inhibition observed around the plug, 1= inhibition observed only directly over the plug and 0=no inhibition observed.

<i>L. edodes</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L	<i>A. mellea</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L
mean	3.96	2.55	1.99	0.93	mean	1.18	1.84	1.20	1.20
std dev	0.11	1.64	0.30	1.01	std dev	0.73	0.27	0.99	0.77
std err	0.04	0.58	0.11	0.36	std err	0.26	0.22	0.35	0.27
Grade	2	2	2	2	Grade	2	2	2	2
<i>A. bisporus</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L	<i>C. nuda</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L
mean	0.76	0.54	1.35	0.95	mean	1.16	3.61	3.75	2.06
std dev	1.64	0.83	0.98	0.77	std dev	0.55	0.58	0.71	0.42
std err	0.58	0.31	0.35	0.27	std err	0.19	0.22	0.25	0.14
Grade	2	2	2	2	Grade	1	2	2	2
<i>A. solidipes</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L	<i>L. sulphureus</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L
mean	0.45	2.13	2.95		mean	1.4			
std dev	0.52	0.41	0.69		std dev	0.94			
std err	0.18	0.14	0.24		std err	0.33			
Grade	1	2	2	0	Grade	1	0	0	0
<i>G. tsugae</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L	<i>G. frondosa</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L
mean		1.88			mean		1.48	0.93	0.73
std dev		0.65			std dev		0.29	0.31	0.37
std err		0.23			std err		0.10	0.11	0.13
Grade	0	2	0	0	Grade	0	2	2	2
<i>G. lucidum</i>	vs S288C	vs MG1655	vs <i>marR</i> R77H	vs <i>gyrA</i> S83L					
mean		0.78	0.38	0.79					
std dev		0.83	1.06	0.85					
std err		0.29	0.38	0.30					
Grade	0	1	1	1					

Minimum Inhibitory Concentration Tests

Inhibition of *E. coli* by antagonists

Extracts of mycelia and growth media from basidiomycete (antagonist) cultures were prepared according to Table 3. MICs for each mycelial and medium extract were recorded relative to the 'reference wells' containing no extract, solely medium and inoculum of the test organism (see Appendix I for MIC plate layout). After the appropriate growth period, MIC values were recorded, where MIC₅₀ is the minimum concentration that gave at least 50% growth inhibition, MIC₉₀ is the minimum concentration that gave at least 90% inhibition, and MIC₁₀₀ is the minimum concentration that gave at least 100% inhibition. A summary of the MIC values for all fungal candidates (antagonists) and test organisms is provided in Figures i- xiv of Appendix II.

Only a few extracts caused full inhibition of test organisms, hence values for 50% inhibition (MIC₅₀), 90% inhibition (MIC₉₀) and 100% inhibition (MIC₁₀₀) were all recorded (Appendix II). Figures 14 and 15 compare the MICs of the different induction treatments as well as the un-induced control for the three *E. coli* test organisms. Figure 14 summarizes the MIC₅₀ results while Figure 15 summarize the MIC₉₀ results. Generally, extracts of culture medium had more inhibitory activity than did extracts of mycelia, with the notable exception of *P. eryngii*, where enhanced MIC₉₀ activity is evident in some mycelial extracts. Also, differences are evident between 'induced' and 'un-induced' treatments, which appear to vary depending on whether mycelial or media extracts were used, and depending on the *E. coli* strain used as a test organism. For example, based on MIC₉₀ values with *P. eryngii* extracts (Figure 15), the most pronounced inhibition was observed with media extracts from EC induction treatment (wild-type, *gyrA*) and the 5AC treatment (*marR*), and, for mycelial extracts, with the LN treatment

(wild-type). From Figure 15 (MIC₉₀), it is also seen that media extracts of 4 un-induced cultures (*L. edodes*, *A. gallica*, *L. sulphureus* and *G. lucidum*) and mycelial extracts of 3 un-induced cultures (*A. mellea*, *P. eryngii* and *G. lucidum*) inhibited all 3 *E. coli* strains tested. In contrast, 8 mycelial extracts and 13 media extracts from induced cultures inhibited all 3 *E. coli* strains tested. It would appear, then, that the 'induction' treatments revealed some inhibitory activity that is not otherwise evident in 'un-induced' culture extracts. Overall, from Figure 15, it appears that the most effective inductions were EC and 5AC.

The different trends observed for the same extract between MIC₅₀ and MIC₉₀ values led me to examine whether MIC₅₀ values have a predictive value for more pronounced inhibition (i.e. MIC₉₀ and MIC₁₀₀). To examine this, I plotted all available MIC₉₀ values against the corresponding MIC₅₀ value (Figure 16a). Similarly, all available MIC₁₀₀ values were plotted against the corresponding MIC₅₀ values (Figure 16b). The statistically significant positive slope evident in these graphs suggest that MIC₅₀ is correlated to MIC₉₀ and MIC₁₀₀. Presumably, if higher concentrations of the active components in extracts were administered, then more cases of MIC₉₀ and MIC₁₀₀ inhibition would be observed.

Table 3. Final concentrations (mg/ml) of crude extracts prepared in 15% ethanol for *E. coli* and *S. cerevisiae* MIC tests. Concentrations vary between different antagonist extracts due to limited yields in some cases. Induction treatments are indicated as LN (Low-Nutrient medium), EC (dead *E. coli* culture), 5AC (5-azacytidine treatment) and UI (un-induced). A single set of MICs were performed, except where indicated by 'R2', corresponding to duplicates performed that contained different concentrations of crude extract for relevant samples. N/A= tests were not performed.

Fungal Source	Mycelia			Medium		
<i>A. solidipes</i>	<i>E. coli</i>	S288C		<i>E. coli</i>	S288C	
LN	38	26		93	161	
EC	28	28		84	84	
5AC	198	198		101	101	
UI	273	173		114	114	
<i>A. mellea</i>			<u>R2</u>			<u>R2</u>
LN	94	16	94	62	60	62
EC	100	113	100	240	198	240
5AC	100	75	100	260	150	260
UI	100	113	100	200	97	200
<i>A. gallica</i>						
LN	32	N/A		33	N/A	
EC	94	N/A		155	N/A	
5AC	86	N/A		122	N/A	
UI	75	N/A		118	N/A	
<i>C. nuda</i>						
LN	62	62		218	218	
EC	103	47		86	86	
5AC	66	66		112	112	
UI	66	66		50	50	
<i>A. bisporus</i>						
LN	38	38		41	41	
EC	25	25		114	114	
5AC	57	57		147	147	
UI	160	160		137	137	
<i>L. edodes</i>						
LN	45	45		55	55	
EC	38	38		22	22	
5AC	38	38		137	137	
UI	60	60		101	101	
<i>H. tessellatus</i>		<u>R2</u>				
LN	38	56	N/A	64	N/A	
EC	38	38	N/A	115	N/A	
5AC	188	118	N/A	113	N/A	
UI	60	60	N/A	115	N/A	

Table 3. cont'd

<i>P. ostreatus</i>						
LN	324		N/A	62		N/A
EC	81		N/A	81		N/A
5AC	38		N/A	90		N/A
UI	114		N/A	114		N/A
<i>P. eryngii</i>						
LN	56		N/A	22		N/A
EC	28		N/A	28		N/A
5AC	75		N/A	25		N/A
UI	88		N/A	25		N/A
<i>G. lucidum</i>						
LN	56		56	35		35
EC	56		56	89		89
5AC	75		75	48		48
UI	47		47	142		142
<i>G. tsugae</i>						
LN	47		N/A	160		N/A
EC	28		N/A	65		N/A
5AC	56		N/A	99		N/A
UI	50		N/A	88		N/A
<i>G. frondosa</i>						
LN	19	<u>R2</u> 57	N/A	115	<u>R2</u> 122	N/A
EC	38	58	N/A	216	179	N/A
5AC	19	19	N/A	150	131	N/A
UI	19	85	N/A	175	150	N/A
<i>L. sulphureus</i>						
LN	83	<u>R2</u> 83	83	87	<u>R2</u> 87	87
EC	53	53	53	186	186	186
5AC	38	156	38	109	133	109
UI	28	28	28	115	115	115
<i>B. edulis</i>						
LN	19		N/A	75		N/A
EC	19		N/A	103		N/A
5AC	19		N/A	103		N/A
UI	19		N/A	113		N/A

MIC₅₀ Mycelial Extracts

a) Wild-type MG1655

b) *marR* R77H

c) *gyrA* S83L

MIC₅₀ Media Extracts

d) Wild-type MG1655

e) *marR* R77H

f) *gyrA* S83L

Figure 14. Comparisons of 'induced' and 'un-induced' cultures of mycelial extracts (left panel) and media extracts (right panel): LN (Low-Nutrient medium), EC (dead *E. coli* culture), SAC (5-azacytidine treatment) and 'un-induced' cultures. Plotted is the inverse MIC₅₀ value [$1/\text{MIC}_{50}$ (mg/ml)] for each extract and each test organism, wild-type MG1655 (a, d), *marR* R77H mutant (b, e), *gyrA* S83L (c, f). Concentration values represent the lowest concentration at which MIC₅₀ was observed. For extracts where an MIC was between two concentration values, the average value was plotted. For extracts tested in multiple experiments, the lower MIC₅₀ is presented.

MIC₉₀ Mycelial

a) Wild-type MG1655

b) *marR* R77H c) *gyrA* S83L

MIC₉₀ Media

d) Wild-type MG1655

e) *marR* R77H f) *gyrA* S83L

Figure 15. Comparisons of 'induced' and 'un-induced' cultures of mycelial extracts (left panel) and media extracts (right panel): LN (Low-Nutrient medium), EC (dead *E. coli* culture), 5AC (5-azacytidine treatment) and 'un-induced' cultures. Inverse of MIC₉₀ [1/ MIC₉₀ (mg/ml)] values are plotted for all basidiomycete (antagonist) for each test organism, MG1655 (a, d), *marR* R77H mutant (b, e) and *gyrA* S83L (c, f). Concentration values represent the lowest concentration at which inhibition was observed. For extracts where an MIC was between two concentration values, the average was plotted. For extracts tested in multiple experiments, the lower concentration was plotted.

a) MIC₅₀ vs MIC₉₀ b) MIC₅₀ vs MIC₁₀₀

Figure 16. General comparison of MIC₅₀ and MIC₉₀ values (a) and MIC₅₀ and MIC₁₀₀ (b). Inverse MICs are plotted [1/MIC (mg/ml)] for all available MIC₉₀ values and the corresponding MIC₅₀ values. A positive slope of $y = 0.206x - 0.0269$ to the regression line of MIC₅₀ vs MIC₉₀ indicates that MIC₅₀ is a reasonable predictor of inhibitory activity of antagonist extracts, likewise for the positive slope, $y = 0.0167x + 0.0273$, of MIC₅₀ vs MIC₁₀₀. Corresponding r and p -values for (a) $r = 0.5008$, $p = 4.9 \times 10^{-9}$ (b) $r = 0.7103$, $p = 3.31 \times 10^{-5}$.

Some differences in susceptibility among the different *E. coli* strains can be observed from Figures 14 and 15, however, such comparisons are more obvious from Figures 17 and 18, where the relative sensitivities to un-induced culture extracts by the three *E. coli* test organism strains are presented. Specifically, I was looking for cases where the ciprofloxacin-resistant strains are more sensitive to an antagonist extract than is the wild-type *E. coli* since such inhibitors would be of considerable value as therapeutic agents. Most of the data showed that mutants were equally, or less susceptible than the wild-type (Appendix II, Figure 17 and 18). Only a few cases were noted where *marR* and *gyrA* mutants were more sensitive to an antagonist treatment than the MG1655 wild-type strain. The most notable case is with mycelial extracts from un-induced cultures of *L. sulphureus* (Figure 18, MIC₉₀). Here, of interest, the extract of medium is apparently non-selective while *marR* and *gyrA* appear more sensitive to the extract of mycelium than does the wild-type *E. coli* strain. A similar trend is seen in the mycelia of *G. frondosa*, where both mutants were found to be more susceptible than the wild-type *E. coli* strain, however the activity of the media extracts was not quantified. There are several examples where one of the two mutants are more sensitive to an extract than the wild-type *E. coli* strain. For example, *gyrA* and *marR* appear to be more sensitive to *A. gallica* mycelium and *G. tsugae* medium extracts, respectively, than is the wild-type *E. coli* strain. These observed cases of enhanced sensitivity by ciprofloxacin-resistant strains warrant additional, focused attention to verify and explore the active compound(s) involved and the mode of activity.

Of all the extracts tested, only media extracts from *L. edodes*, *C. nuda* and *L. sulphureus* and the mycelia extract from *A. solidipes* exhibited full inhibition (i.e. MIC₁₀₀) of all *E. coli* strains

(Fig. 19). Media extracts of 5AC- and EC-treated *L. edodes* and *C. nuda* cultures exhibited MIC₁₀₀ values, all *L. sulphureus* extracts exhibited MIC₁₀₀ values, and mycelial extracts of 5-AC-treated *A. solidipes* cultures exhibited MIC₁₀₀ values. These fungal species are, therefore, excellent candidates for exploring further leads of an antimicrobial inhibitor(s).

a) MIC₅₀ for Mycelial extracts of 'un-induced' cultures b) MIC₅₀ for Media extracts of 'un-induced' cultures

Figure 17. A summary of activity of mycelial (a) and media (b) extracts of 'un-induced' cultures for all fourteen fungal antagonists against the three bacterial strains wild-type MG1655 (blue bars), *marR* R77H (orange bars) and *gyrA* S83L (grey bars). Inverse MIC of the concentrations at MIC₅₀ [1/ MIC₅₀ (mg/ml)] are plotted.

a) MIC₉₀ for Mycelial extracts of 'un-induced' cultures b) MIC₉₀ for Media extracts of 'un-induced' cultures

Figure 18. A summary of activity of mycelial (a) and media (b) extracts of 'un-induced' cultures for all fourteen fungal antagonists against the three bacterial strains, wild-type MG1655 (blue bars), *marR* R77H (orange bars) and *gyrA* S83L (grey bars). Inverse MIC of the concentrations at MIC₉₀ [1/ MIC₉₀ (mg/ml)] are plotted.

Figure 19. Data of concentrations of extracts that yielded MIC₁₀₀ values against *E. coli*. Comparisons are shown of medium extracts from *L. edodes*, *C. nuda*, *L. sulphureus* and the mycelia of *A. solidipes*. Extracts were of 'induced' and 'un-induced' cultures [LN (Low-Nutrient medium), EC (dead *E. coli* + medium), SAC (5-azacytidine treatment) and un-induced cultures]. Inverse MIC concentrations of the fungal extracts at MIC₁₀₀ [1/ MIC₁₀₀ (mg/ml)] are plotted for *marR* R77H and *gyrA* S83L mutants, and for wild-type MG1655 *E. coli* strains. Concentration values represent the lowest concentration at which inhibition was observed. For extracts where an MIC was between two concentration values, the average was plotted. For extracts tested in multiple experiments, the lower concentration was plotted.

Inhibition of *Saccharomyces cerevisiae* by antagonists

Growth of *S. cerevisiae* was inhibited in top agar bioassays by *L. edodes*, *C. nuda*, *A. bisporus*, *A. mellea*, *A. solidipes* and *L. sulphureus* (Table 2). The largest inhibition zone was seen with *L. edodes*, with an average inhibition diameter of 3.96 cm, followed by *A. mellea* and *C. nuda* with average inhibition diameters of 1.18 cm and 1.04 cm, respectively. Similarly, MIC₅₀ tests with *S. cerevisiae* revealed highest activity by *L. edodes* extracts, followed by *L. sulphureus* and *C. nuda*. *A. bisporus*, *A. solidipes* and *A. mellea*, did not inhibit *S. cerevisiae* in MIC tests. *L. sulphureus* was the only fungal antagonist tested for which antifungal activity was evident with extracts from both induced and un-induced cultures. Media extracts from these three fungal candidates were generally more active than the mycelial extracts (Fig. 20). As in the antibacterial assays, some effect of 'induction' treatments is evident, most notably the

mycelium extract of LN-treated *L. sulphureus* was very active against yeast. By comparison, the relatively low antibacterial activity may suggest that the inhibitor compound in this extract has specificity to fungi, although this needs to be explored further. Overall, the medium extracts from these 3 species display interesting antifungal activity that is worthy of further study.

a) MIC₅₀ of antagonist against *S. cerevisiae*

b) MIC₁₀₀ of antagonist against *S. cerevisiae*

Figure 20. Mycelial and media extracts of 'induced' and 'un-induced' cultures [LN (Low-Nutrient medium), EC (dead *E. coli* + medium), 5AC (5-azacytidine treatment) and un-induced] tested against *S. cerevisiae*. Inverse MIC of the concentrations [1/ MIC (mg/ml)] at MIC₅₀ (a) and MIC₁₀₀ (b) are plotted for species demonstrating antifungal activity in MIC tests.

Relationship between Top Agar and Minimum Inhibitory Concentration Tests

I tested whether there is a correlation between the top agar and MIC assays, as depicted in Figure 21. This graph plots inhibition zone diameters (Table 3) against the respective MIC₅₀ concentrations. Figure 21a indicates that there is, overall, a slight positive slope in the regression line, indicating that antagonists that are active in top agar bioassays may ultimately produce inhibitors that are active in MIC assays, although this correlation is not statistically significant ($p=0.8$). Figure 21b provides a visual representation of regression lines for each species, and shows that positive correlations are prominent for *A. solidipes*, *A. mellea*, *G. frondosa* and *C. nuda*. Based on this analysis, it appears that the top agar bioassay may not be a good predictor of MIC activity.

a) General trend

b) Individual fungi

Figure 21. Correlation between mean inhibition diameter in top agar bioassay and the inverse of the mean MIC_{50} value [$1/\text{MIC}$ (mg/ml)]. (a) shows the general trend for all fungal extracts, $y = 0.0128x + 0.3899$, $r = 0.0341$, $p = 0.834$ and (b) shows the data for each specific fungal extract.

Biostatic vs biocidal activity of extracts

Generally, media extracts exhibited more pronounced antimicrobial activities than the mycelial extracts, as evident by the prevalence of MIC₁₀₀ readings, that were observed mostly with media extracts (Figure 19 for antibacterial, and Figure 20 for antifungal). This suggests that, when produced, antimicrobial compounds are excreted by the basidiomycete antagonists, rather than sequestered as intracellular or surface-bound agent(s). To determine whether inhibitors yielding MIC₁₀₀ values were biocidal or biostatic, I transferred the contents of wells that registered MIC₁₀₀ values into the appropriate medium to dilute the inhibitory compounds, and looked for the resumption of growth by the test organism after further incubation. The results of these assays are given in Table 4. Upon dilution, bacterial and/or fungal growth resumed within 24 hrs when the contents of wells containing fully inhibitory concentrations of *L. edodes* and *C. nuda* media extracts were tested. The same samples, when plated directly on an appropriate agar medium also resulted in lawns of bacterial/fungal growth after two to four days of incubation. This indicates that these extracts from *L. edodes* and *C. nuda* contain bacteriostatic and/or fungistatic compounds. Similarly, the contents of wells with *L. sulphureus* extracts in which growth by *S. cerevisiae* was fully inhibited, contained live cells, consistent with a fungistatic inhibitor. In contrast, mycelial extracts from the 5-AC-treated cultures of *A. solidipes* did not demonstrate bacterial growth once diluted from the respective MIC₁₀₀ wells in neither agar plates nor liquid broth. The inhibitory compound(s) from *A. solidipes* can, therefore, be considered as bactericidal. These tests indicated that most of the inhibitors detected were biostatic, at least at the concentrations tested. It is interesting that the prominent bactericidal extract from *A. solidipes* was also unusual in being localized in the

mycelium and was not abundant in the medium extract. In most cases, extracts behaved the same way against all *E. coli* strains; where extracts were bacteriostatic, they were bacteriostatic against all three *E. coli*, and likewise for bactericidal extracts. The exception was media extracts of *L. sulphureus* that were bacteriostatic or bactericidal, depending on the type of induction treatment used; both EC and 5-AC-treated *L. sulphureus* cultures yielded media extracts containing bactericidal compound(s), while LN-treated and un-induced (UI) extracts were bacteriostatic. This is interesting since it indicates that this *L. sulphureus* may produce different inhibitors depending on growth conditions. The observations may also be explained if the bactericidal extracts act in a dose-dependent manner, as evident from agar inoculation experiments, wherein wells containing crude extract concentrations that were lower than the relative MIC₁₀₀ (test well), exhibited low colony numbers of microbial growth, and colony numbers increased with decreasing extract concentrations (data not shown).

Table 4. Mechanism of inhibitory action of extracts of indicated treatments, LN=low-nutrient medium, EC= spent *E. coli* addition, 5AC=5-azacytidine addition and UI=un-induced, observing full inhibition (i.e. MIC100) on respective microbes.

	MIC100 mycelium	MIC 100 medium	biostatic/biocidal
<i>L. edodes</i>			
<i>marR</i>	ND	EC/5AC	Bacteriostatic
<i>gyrA</i>	ND	EC/5AC	Bacteriostatic
MG1655	ND	EC/5AC	Bacteriostatic
S288C	ND	EC/5AC/UI	Fungistatic
<i>C. nuda</i>			
<i>marR</i>	ND	EC/5AC	Bacteriostatic
<i>gyrA</i>	ND	EC/5AC	Bacteriostatic
MG1655	ND	EC/5AC	Bacteriostatic
S288C	LN/EC	LN/EC/5AC	Fungistatic
<i>A. solidipes</i>			
<i>marR</i>	5AC	ND	Bactericidal
<i>gyrA</i>	5AC	ND	Bactericidal
MG1655	5AC	ND	Bactericidal
S288C	ND	ND	ND
<i>L. sulphureus</i>			
<i>marR</i>	ND	LN/EC/5AC/UI	LN ^s EC ^c 5AC ^c UI ^s
<i>gyrA</i>	ND	LN/EC/5AC/UI	LN ^s EC ^c 5AC ^c UI ^s
MG1655	ND	LN/EC/5AC/UI	LN ^s EC ^c 5AC ^c UI ^s
S288C	LN/EC	LN/EC/5AC	Fungistatic

Superscript c= biocidal, superscript s= biostatic

SECTION IV: Discussion

Fungi are known to produce secondary metabolites with various types of activity, including antibacterial and antifungal activities. The purpose of this study was to evaluate a small subsample of medicinal and/or edible mushrooms for inhibitory activity against the microbes *Escherichia coli* and *Saccharomyces cerevisiae*. In addition, I set out to test the relative susceptibility of antibiotic-resistant *E. coli* to identify antibacterial candidates effective against ciprofloxacin-resistant bacteria. Two types of tests were done to deduce the activity of the fungi. First, a top agar *in-vivo* test was done which involved determining inhibitory activity by live mycelia against the *E. coli* and *S. cerevisiae* test organisms. In this case, antimicrobial activity was indicated by inhibition zones formed around the mycelial plugs, where test organism growth was arrested. The second method used involved minimum inhibitory concentration (MIC) tests, wherein crude extracts were prepared from chemically- and environmentally-induced fungal (antagonist) cultures and evaluated in broth-dilution (MIC) tests. The effectiveness of induction treatments can be inferred in comparison to MIC values from un-induced cultures.

The top agar bioassays are simpler and faster to perform and appear to serve as a reasonable preliminary test to establish antibacterial and antifungal activity of the fungi. The top agar test also can also be used to verify data observed in MIC tests. However, fungi that did not test positive in the top agar bioassays may still produce antimicrobial compounds as indicated by MIC assays. In some cases, discrepancies between the two assays may relate to inclusion, or lack of, an appropriate induction treatment. For example, *A. gallica* and *P. eryngii* did not demonstrate inhibition in the top agar bioassays, but did have activity in the MIC tests;

A. gallica demonstrated MIC₉₀ of mutant and wild-type *E. coli* at higher media extract concentrations and *P. eryngii* demonstrated low MIC concentration that steadily inhibited growth of *E. coli* by 50%, even at higher concentrations. In addition, *L. sulphureus* inhibited only *S. cerevisiae* in the top agar assays but showed strong antimicrobial inhibition in the MIC tests against both *S. cerevisiae* and *E. coli*, with full inhibition of *S. cerevisiae* by the extracts from induced cultures and of *E. coli* by all extracts, including the un-induced. The top agar results may also provide a correlation to inhibitory concentrations; fungi with larger inhibition zone in the top agar assays tended to have lower MIC's (i.e. require less extract to cause inhibition), as seen in Figure 21b. Top agar assays also apparently give some indications of different modes of inhibitory activity. For example, some fungi inhibited microbial growth solely around the plugs (i.e. grade= 1, Table 2) while others produced a relatively large clear zone (halo) around the plug (i.e. grade= 2, Table 2). The former, restricted inhibition may be expected for antagonists that have antimicrobial activity in the mycelium whereas large clear zones may indicate a release of antimicrobial compounds into the surrounding environment. In fact, most of the fungi that were categorized with grade of 2 in top agar assays did, indeed, have more activity in the media extracts. *L. edodes*, *C. nuda*, *A. solidipes* and *A. mellea* were all grade=2 with prominent clear zones in top agar assays and all yielded MIC₉₀ and/or MIC₁₀₀ values in MIC tests. Similarly, *A. bisporus*, *G. frondosa* and *G. tsugae* were grade=2 and had more activity in the media than the mycelial extracts (Table 2 and Figure 14 and 15). Interestingly, *A. solidipes* and *G. lucidum* demonstrated counterintuitive effects. *A. solidipes*, was assigned a grade=2 in top agar assays, and yet demonstrated MIC₁₀₀ with bactericidal activity with mycelial extracts (Figure 20 and Table 4) from cultures treated with 5-azacytidine. *G. lucidum*, was assigned

grade=1 in the top agar assays but demonstrated relatively high antibacterial activity with the media extracts. This latter result with *G. lucidum* may be due to the induction treatments used on the cultures, causing the mycelia to excrete more or novel inhibitory compounds. Similarly, *A. solidipes* mycelial activity may be due to induction treatments causing deposition of inhibitors in the cell wall or internal cellular compartments.

Several previous studies have investigated the antibacterial and antifungal activity of basidiomycete fungi (review in Alves *et al.*, 2012). For my study, I selected candidate fungal antagonists that were available and of known edibility and/or medicinal properties and therefore regarded as safe for human consumption/use. Secondary metabolite induction with epigenetic modifiers was also investigated in a previous study by Williams *et al.* (2008). To the extent of our knowledge, induction treatments have not been used with basidiomycete fungi in an attempt to produce more or different secondary metabolites. Generally, I found that the induction treatments did not appear to greatly increase antibacterial activity. This is seen in Figure 14 where comparative activity at MIC₅₀ of the induced and un-induced antagonists are plotted and activity between the treatments was generally equivalent among the fungal antagonists. However, a few fungi had higher antimicrobial activity in the extracts from induced cultures (Figures 14, 17, 23). In particular, EC and 5AC-treatments were apparently most effective in this regard. In contrast, LN was not an effective induction treatment; fungi that demonstrated antibacterial activity in the LN extract generally had higher activity in the un-induced control extracts.

It should be noted that the induction methods chosen may not be optimal and that there are potential unlimited other conditions that could be tested, including variations in types

of induction (e.g. different chemicals), timing of induction, concentrations of inducing agents, etc. For example, the concentration of 5-azacytidine in 5AC treatments was chosen based on the protocol of Williams *et al.* (2008). In their study, they used a different concentration of 5-AC for each fungus species (mould) tested. They chose their concentrations by identifying a 5-AC MIC concentration for each fungus and used a concentration that was 10-fold lower than the MIC value. The lowest concentration of 5-AC, 0.1 μ M, that Williams *et al.* (2008) used in their study was chosen for the 5-AC treatment protocol in my study but other concentrations or treatment times or durations may result in more pronounced induction. Performing 5-AC MIC's for the fungal antagonists used in this study is logistically difficult, but possible. Doing MICs with filamentous fungi can be challenging since the mycelia need to be fragmented into uniform Colony Forming Units (CFU's) and then enumerated and preserved by freezing prior to use in MICs. Being slow-growers, quantifying CFU's requires 2 – 10 days incubation. Not all fungi survive the freezing storage process. Hence, due to time constraints, the lowest concentration of 5-AC, 0.1 μ M, used by Williams *et al.* (2008) was arbitrarily chosen. Similarly, the spent *E. coli* medium induction protocol was also based on the Williams *et al.* (2008) study. While this treatment seemed to enhance production of antimicrobials from the fungal antagonists in many cases, in general, several variations on this protocol are possible – some of which may be more efficacious. For example, a similar induction method could utilize natural bacterial and/or fungal competitors to the antagonistic fungi that may better trigger a defense mechanism by the antagonist. Finally, as it seems that the low-nutrient medium was not very effective in causing enhanced antimicrobials production, perhaps the composition of the low-nutrient medium could be optimized to stress the basidiomycete antagonist. It is widely acknowledged

that controlling culture conditions and modifying medium composition can dramatically enhance the production of biologically active compounds (Walkowiak and Subramaniam, 2014; Barborakova *et al.*, 2012; Lin and Sung, 2006). Different temperature, pH and quantities or compositions of carbon, nitrogen, salts, etc, could be tested as potential inducers in the medium.

Knowing which specific induction factors increase antimicrobial activity may also be helpful for compound identification and quantification. For example, our finding that 5-AC is generally a good inducer for selected antagonists suggests that antimicrobial pathways are modulated by methylation-associated gene controls. It may be possible to identify methylated tracts of DNA and to infer the genes involved in production of specific inhibitors. Otherwise, bioassay-guided fractionation can be used to identify antimicrobials (Galván *et al.*, 2008). Once a compound is identified, each of the ingredients and/or environmental factors that are known to stress the culture into producing antibacterial compounds can then be tested in different amounts to assess more efficiently whether production of the compound can be upregulated.

In addition to secondary metabolites, fungi are known to produce and secrete enzymes and small peptides that may be important for the breakdown of organic matter, for signalling, for host infection and as antimicrobials (Hankin and Anagnostakis, 1975). In fact, *Trichoderma* species are well studied for their ability to produce chitinases, glucanases and proteases that are believed to be involved in mycoparasitism (Benitez *et al.* 2004). These enzymes are released by *Trichoderma* spp. when contact is made with a host and work by degrading the cell wall of other fungi. Benitez *et al.* (2004) mention an induction of gene expression of chitinase when *Trichoderma* strains directly contact a fungal cell wall. So, while antimicrobial secondary

metabolites should be identified from fungal antagonists examined in this study, perhaps studying the enzymes, and enzyme inductions, would be of great interest as well.

Overall, almost all the extracts tested in this study exhibited low levels of inhibitory activity (MIC_{50}) towards bacteria and/or fungi and some exhibited stronger levels of inhibition (MIC_{90} or MIC_{100}). Most extracts of *L. edodes*, *C. nuda*, *A. gallica*, *A. solidipes*, *A. mellea*, *L. sulphureus* and *G. lucidum* yielded MIC_{90} values - with activity observed more so in the media extracts (Figures 14 and 15). It should be noted, however, that extract concentrations of the medium extracts were generally higher than mycelial extracts, and this may limit the apparent activity of the mycelial crude extracts. In addition, antimicrobial activity was prevalent in some extracts from induced cultures. Therefore, this study identifies interesting antimicrobial leads, and reveals that such leads should be carefully examined for antimicrobial potential. It is unknown from my study whether extracts with identifiable antimicrobial activity contain small quantities of very effective inhibitor(s) or relatively large quantities of weak inhibitors. Nevertheless, that nearly all extracts had antimicrobial activity from the relatively small subset of edible basidiomycetes I tested is noteworthy, and indicates that such fungi are a potentially good source of bioactive compounds. Furthermore, investigation of the relatives of species that were found to produce antimicrobials in my study may be worthwhile.

Based on the results from my study, *L. edodes*, *C. nuda*, *A. solidipes*, *A. mellea*, *G. lucidum* and *L. sulphureus* are good candidates for production of antimicrobials. In top agar assays, *L. edodes*, *A. bisporus*, *A. solidipes* and *C. nuda* gave large inhibition diameters with the bacteria and yeast tested and extracts of all but *A. bisporus* yielded full inhibition of bacterial and fungal growth in MIC assays. These findings are consistent with previous studies in which *L.*

edodes, *A. bisporus* and *C. nuda* were reported to have high antibacterial activity yields when compared to various other fungal species (Alves *et al.* 2012; Hatvani, 2000; Hirasawa *et al.*, 1999). The bacteriostatic activity of *L. edodes* and *C. nuda* evident from my study gives additional insight on the mechanism of antibacterial activity as the bacterial and fungal cells appeared to survive the exposure to crude extracts and subsequently grow, despite being completely inhibited in the sample MIC wells. The extracts were bacteriostatic such that, when contents were spread onto plates, the numbers of colonies were constant among MIC wells with full inhibition (i.e. MIC₁₀₀) and MIC wells with MIC₉₀ and MIC₅₀, meaning that crude extracts did not destroy the cells, only inhibited their growth (Pankey and Sabath, 2004). In contrast, extracts of *A. solidipes* and *L. sulphureus* demonstrated bactericidal activity. Bactericidal activity is of obvious interest for further research.

Previous studies found high antibacterial activity in the extracts from on *Armillaria* spp. (Donnelly *et al.*, 1985; Anderson *et al.*, 1979). However, these previous studies do not specify which species of *Armillaria* were investigated since before the 1990's, *A. gallica*, *A. solidipes* and *A. mellea* were all taxonomically combined into the species *A. mellea*. Now that the taxa have been separated into different species, activity of the specific species can be established- in this case, all species demonstrated antibacterial activity, with *A. mellea* and *A. gallica* demonstrating MIC₉₀ values and *A. solidipes* demonstrating MIC₁₀₀ inhibition and bactericidal activity. Sample wells with full bacteria inhibition of *A. solidipes* did not re-colonize in fresh medium or on agar plates where inhibitor(s) had been diluted. To my knowledge, there have not been studies on whether *Armillaria* spp. produce antifungals. My study demonstrated

antifungal properties of *A. mellea* and *A. solidipes* in the top agar assays, however inhibitory concentrations could not be demonstrated from the MIC assays.

Of interest, *G. lucidum* fungi demonstrated positive top agar results against *E. coli*, and MIC₉₀-level inhibition at higher concentrations. Antibacterial activity of *G. lucidum* was previously tested by Quereshi *et al.* (2010) with disc diffusion assays. Different extracts demonstrated antibacterial activity against several bacteria, with the second lowest MIC being against *E. coli*, out of the bacteria they tested. MIC assays performed with *G. lucidum* in this study did not demonstrate antifungal properties against *S. cerevisiae*, however previous studies found that *G. lucidum* produced a protein, ganodermin, which inhibited mycelial growth of ascomycetes *Botrytis cinerea*, *Fusarium oxysporum* and *Physalospora piricola* (Wang and NG, 2006). This could indicate that ganodermin is not produced by the strain used in my study or that there is specificity of this inhibitor such that brewer's yeast is not effected.

Another particularly interesting candidate revealed in my studies is *P. eryngii*. Previous studies have noted that extracts of *P. eryngii* have antibacterial activity against select bacteria, however mechanisms or mode of action were not investigated (Akyuz and Kirbag, 2009; Uzen *et al.* 2004). Akyuz *et al.* (2009) performed disc diffusion assays with *P. eryngii* extracts, and inhibition zone diameter ranged from 7.0- 22.0 mm on various bacteria tested, specifically 8.0- 10.0 mm against *E. coli* (100µg on a 6 mm disc). Activity varied greatly among *P. eryngii* extracts using different solvents and between different bacterial species. The present study shows that relatively low concentrations of *P. eryngii* extracts inhibit growth of *E. coli* by 50%, even at higher concentrations. This study also shows that induction of *P. eryngii* cultures increased the antimicrobial activity in extracts (Figure 14 and 15). Antibacterial findings on *P. eryngii* in this

study are consistent with the previous findings in that *P. eryngii* activity was not a strong antibacterial, and builds on our understanding of the mechanisms of antibacterial action by *P. eryngii* extracts.

This study indicates that *L. sulphureus* is an excellent candidate for antimicrobial investigation and another good example of how inductions of fungal cultures affect antimicrobial activity. In top agar assays, *L. sulphureus* only inhibited *S. cerevisiae* but showed strong antimicrobial inhibition in the MIC tests against both *S. cerevisiae* and *E. coli* strains tested. Extracts demonstrated full inhibition of *S. cerevisiae* by the extracts from induced cultures and of *E. coli* by all extracts, including extracts from the un-induced cultures. Furthermore, my results indicate that *L. sulphureus* can be induced to produce either biostatic or biocidal inhibition. Extracts from cultures treated with killed-*E. coli* filtrate as well as the cultures treated with 5-azacytidine were bactericidal while the extracts from un-induced cultures and the cultures treated with low-nutrient medium were bacteriostatic (Table 4). This indicates that *L. sulphureus* can possibly be induced to make new (or more) compounds. MIC's for *L. sulphureus* in this study ranged from 2.71-46.5 mg/mL against *E. coli* and 10.9-27.3 mg/mL for *S. cerevisiae*. Inhibition zones in top agar bioassays averaged 0.88 cm against *S. cerevisiae* but no inhibition zones for *E. coli*. The antimicrobial MIC findings are consistent with the findings in previous studies. Sinanoglou *et al.* (2015) found antifungal and antibacterial activity in hexane and chloroform extracts of *L. sulphureus* and Turkoglu *et al.* (2007) found activity against Gram-negative and Gram-positive bacteria in ethanol extracts. Both studies demonstrated activity against *E. coli* with MIC values in the range of 2.0 ± 0.07 mg/mL (hexane extract) and 1.56 ± 0.02 mg/mL (chloroform) (Sinanoglou *et al.* 2015). Agar well diffusions

against *E. coli* resulted in 10 mm inhibition zone, similar to my study. That *L. sulphureus* did not exhibit antibacterial activity *in-vivo* with the top agar assays, but was active in the un-induced control extract, may indicate that growth phase, liquid culture, or handling of the culture may influence the ability of this species to produce secondary antimicrobial metabolites. Another possible explanation, is that the top agar bioassays were not optimal for testing *L. sulphureus*. In some cases, where the top agar did not completely cover the inoculum plug, the mycelium that was not covered was able to grow outwards on the top agar, and produced a zone where *E. coli* growth was then inhibited. This phenomenon was also seen in many *S. cerevisiae* top agar tests with *G. lucidum*, where inhibition zones were larger when the mycelia were unsubmerged and able to grow on the top agar. This may be due to aerobic vs hypoxic conditions in submerged cultures.

Antifungal MIC's were only performed with fungal extracts that were antifungal-positive in the top agar bioassays against *S. cerevisiae*. Six antagonist fungi demonstrated antifungal activity against *S. cerevisiae*: *L. edodes*, *C. nuda*, *A. bisporus*, *A. mellea*, *A. solidipes* and *L. sulphureus*. Only three candidates of the six fungi tested demonstrated antifungal activity in MIC assays, *L. edodes*, *C. nuda* and *L. sulphureus*. Decreased growth(MIC₅₀) was difficult to detect in the *S. cerevisiae* assays as the cells would sediment to the bottom of the wells, and absorbance readings would not accurately detect optical density differences between the wells with intermediate growth, hence for much of the *S. cerevisiae* MIC₅₀ were recorded MIC₅₀ > x, x= corresponding to well with greatest extract concentration. MIC₁₀₀ were easily detected with yeast, however, and plotted in Figure 24, where *L. edodes*, *C. nuda* and *L. sulphureus* are presented with activity in both the mycelia and media extracts.

Now that antimicrobial activity has been established for the basidiomycete fungi examined in my study, several measures can be taken to optimize activity, mode of action can be investigated as well as identifying active compounds within the extracts. To optimize antimicrobial activity in the crude extracts, the induction treatments can be investigated and ameliorated; as mentioned above, the concentration of 5-azacytidine can be optimized for the different fungi to yield an extract with maximum activity, cell filtrates other than *E. coli* could be investigated as an induction treatment and finally, optimizing a composition of ingredients in the low-nutrient medium may induce increased antimicrobial activity. To gain further insight on mode of antibacterial and antifungal action gene deletion arrays (GDA) with *E. coli* and *S. cerevisiae* libraries, respectively, can be done (Darvishi *et al.* 2013). In GDA assays, deletion mutants that are super sensitive to the compound/extract provide information of a chemical-genetic interaction. From this inference on the mode of action of the antimicrobial can be obtained. Active compounds produced by fungi can be identified through fractionation and analytical chemistry techniques. Bioassay-guided fractionation is a conventional method to isolate and identify unknown compounds. This method involves separating compounds by fractionation, e.g. column chromatography, and testing each fraction using antimicrobial assays. Active compounds can then be identified using NMR spectroscopy and/or mass spectroscopy. Alternatively, comparative metabolomics could also be used to identify active compounds in specific extracts which can also narrow a list of active compounds by identifying members of known compound families by matching their fragmentation patterns. Once the active compounds are known, they can then be tested individually on various bacterial and fungal pathogens, in different concentrations to determine MIC of the specific compound.

In conclusion, my study demonstrates antimicrobial activity in extracts of edible and medicinal fungi and that enhanced antimicrobial activity can be induced with different chemical and environmental cues. Extracts of other Basidiomycetes should be investigated to identify compounds to target antibiotic resistance.

SECTION V: References

- Adachi Y, Ohno N, Ohsawa M. 1990. Change of biological activities of (1-3)-beta-D-glucan from *Grifola frondosa* upon molecular weight reduction by heat treatment. Chemical and Pharmaceutical Bulletin; **38**:477-481.
- Akyuz M. and Kirbag, S. 2009. Antimicrobial activity of *Pleurotus eryngii* var. *ferulae* grown on various agro- wastes. EurAsian Journal of BioSciences; **3**: 58-63.
- Alakomi HL., Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. 2000. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. Applied and Environmental Microbiology; **66**: 2001-2005.
- Alanis AJ. (2005). Resistance to Antibiotics: Are We in the Post-Antibiotic Era? Archives of Medical Research; **36**: 697–705.
- Alekshun MN, and Levy SB. 1999. The mar regulon: multiple resistance to antibiotics and other toxic chemicals. Trends in Microbiology; **7**:410-413.
- Alves MJ, Ferreira I, Dias J, Teixeira V, Martins A, Pintado M. 2012. A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Med; **78**: 1707–1718.
- Amagasse H. 1987. Treatment of hepatitis B patients with *Lentinus edodes* mycelia. In: New Trends in Peptic Ulcer and Chronic Hepatitis. Part II. Chronic Hepatitis. Princeton: Excerpta Medica; **3**: 16-21.

American Academy of Microbiology. 2009. Antibiotic Resistance: An Ecological Perspective on an Old Problem. A Report from the American Academy of Microbiology.

American Public Health Association. 2008. Diarrhea, acute. In DL Heymann, ed., Control of Communicable Diseases Manual, 19th ed., pp. 179-195. Washington, DC: American Public Health Association.

Anderson JB and Ullrich RC. 1979. Biological species of *Armillaria mellea* in North America. *Mycologia*; **71**: 402–414.

Anderson JB, Bailey SS, Pukkila PJ. 1989. Variation in ribosomal DNA among biological species of *Armillaria*, a genus of root-infecting fungi. *Evolution*; **43**: 1652–1662.

Barborakova Z, Labuda R, Haubl G, Tancinova D. 2012. Effect of Glucose Concentration and Growth Conditions on the Fungal Biomass, pH of Media and Production of Fumagillin by A Non-Pathogenic Strain *Penicillium scabrosum*. *Journal of Microbiology, Biotechnology Food Sciences*; **1**: 466-477.

Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR. 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chemical Toxicology*; **46**: 2742-2747.

Beltran-Garcia MJ, Estarron-Espinosa M, Ogura T. 1997. Volatile Compounds Secreted by the Oyster Mushroom (*Pleurotus ostreatus*) and Their Antibacterial Activities. *Journal of Agricultural and Food Chemistry*; **45**: 4049–4052.

- Bender S, Dumitrache CN, Backhaus J, Christie G, Cross RF, Lonergan GT, Baker WL. 2003. A case for caution in assessing the antibiotic activity of extracts of culinary-medicinal Shiitake mushroom [*Lentinus edodes* (Berk.) Singer] (Agaricomycetideae). *International Journal of Medicinal Mushrooms*; **5**: 31–35.
- Benedict RG and Brady LR. 1972. Antimicrobial Activity of Mushroom Metabolites. *Journal of Pharmaceutical Sciences*; **61**: 1820-1821.
- Benitez T, Rincon AM, Limon MC, Codon AC. 2004. Biocontrol mechanisms of Trichoderma strains. *International Microbiology*; **7**:249-260.
- Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, and Piddock LJV. 2015. Molecular Mechanisms of Antibiotic Resistance. *Nature Reviews: Microbiology*; **13**: 42-51.
- Brown GB and Weliky VS. 1953. The Synthesis of 9-P-N-Ribofuranosylpurine and the Identity of Nebularine. *Journal of Biological Chemistry*; **204**: 1019-1024.
- Chang ST, Mshigeni KE. 2001. Mushroom and Human 13. Health: their growing significance as potent dietary supplements. University of Namibia, Windhoek, Namibia. 1-79.
- Chang R. 1996. Functional Properties of Edible Mushroom. *Nutrition Reviews*; **54**: S91-S93.
- Chibata I, Okumura K, Takeyama S, Kotera K. 1969. Lentinacin: A New Hypocholesterolemic Substance in *Lentinus edodes*. *Specialia Experientia*; **25**: 1237-1238.
- Darvishi E, Omid M, Bushehri AA, Golshani A, Smith ML. 2013. The antifungal Eugenol perturbs dual aromatic and branched-chain amino acid permeases in the cytoplasmic membrane

- of yeast. *PLOS ONE*; **8**: e76028. doi:10.1371/journal.pone.0076028.
- Donnelly DM, Abe F, Coveney D, Fukuda N, O'Reilly J, Polonsky J, Prangé T. 1985a. Antibacterial Sesquiterpene Aryl Esters from *Armillaria mellea*. *Journal of Natural Products*; **48**: 10–16.
- Donnelly DMX, Quigley PF, Coveney DJ, Polonsky J. 1985b. Two new sesquiterpene esters from *Armillaria mellea*. *Phytochemistry*; **26**: 3075-3077.
- Dulger B, Ergul CC, Gucin F. 2002. Antimicrobial activity of the macrofungus *Lepista nuda*. *Fitoterapia*; **73**: 695–697.
- Elmastas M, Isildak O, Turkekul I, Temur N. 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*; **20**: 337–345.
- Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, Bradford PA, Angulo FJ, Hinrichs SH. 2000. Ceftriaxone-Resistant Salmonella Infection Acquired by a Child from Cattle. *The New England Journal of Medicine*; **342**: 1242-1249.
- Fons F, Roumestan C, Rapior S. 2005. Biodiversité, règne fongique et thérapeutique. 1. Connaissances actuelles et perspectives. *Annales de la Société d'Horticulture et d'Histoire Naturelle de l'Hérault*; **145**: 87-89.
- Fothergill AW. 2012. Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods. *Interactions of Yeasts, Moulds, and Antifungal Agents*. Chapter 2: 65-74.

- Galván IJ. 2016. Elucidating mechanisms of action of bioactive substances by chemical-genetic profiling. PhD Thesis, Carleton University. Ottawa, Ontario, Canada.
- Galván IJ, Mir-Rashed N, Jessulat M, Atanya M, Golshani A, Durst T, Petit P, Treyvaud Amiguet V, Boekhout T, Summerbell R, Arnason JT, Smith ML. 2008. Antifungal and antioxidant activities of the phytomedicine Pipsissewa, *Chimaphila umbellata*. *Phytochemistry*; **69**: 738-746.
- Gardiner DM, Kazan K, Manners JM. 2009a. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. *Fungal Genetic Biology*; **46**: 604–613.
- Ghannoum MA and Rice LB. 1999. Antifungal Agents: Mode of Action, Mechanisms of Resistance, and Correlation of These Mechanisms with Bacterial Resistance. *Clinical Microbiology Review*; **12**: 501–517.
- Ha TB, Gerhauser C, Zhang WD, Ho-Chong-Line N, Fouraste I. 2000: New lanostanoids from *Ganoderma lucidum* that induce NAD(P)H: quinone oxidoreductase in cultured hepatic 7 murine hepatoma cells. *Planta Medica*; **66**: 681- 684.
- Hankin L and Anagnostakis SL. 1975. The Use of Solid Media for Detection of Enzyme Production by Fungi. *Mycologia*; **67**: 597-607.
- Hatvani N. 2001. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. *International Journal of Antimicrobial Agents*; **17**: 71-74
- Hearst R, Nelson D, McCollum G, Millar BC, Maeda Y, Goldsmith CE, Rooney PJ, Loughrey A, Rao

- JR, Moore JE. 2009. An examination of antibacterial and antifungal properties of constituents of Shiitake (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms. *Complementary Therapies in Clinical Practice*; **15**: 5-7.
- Hirasawa M, Shouji N, Neta T, Fukushima K, Takada K. 1999. Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). *International Journal of Antimicrobial Agents*; **11**: 151-157.
- Hobbs C. 2002. Medicinal Mushrooms. An Exploration of Tradition, Healing, & Culture (Herbs and Health Series).
- Hur JM, Yang CH, Han SH, Lee SH, You YO, Park -C, Kim KJ. 2004. Antibacterial effect of *Phellinus linteus* against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia*; **75**: 603-605.
- Ishikawa NK, Kasuya MCM, Vanetti MCD. 2001. Antibacterial activity of *Lentinula edodes* grown in liquid medium. *Brazilian Journal of Microbiology*; **32**: 206-210
- Iwalokun BA, Usen UA, Otunba AA, and Olukoya DK. 2007. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *African Journal of Biotechnology*; **6**: 1732-1739.
- Jones K. 1995. Shiitake: The Healing Mushroom. Inner Traditions Int. 52-74.
- Kalač P. 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: *A Review: Food Chemistry*; **113**: 9-16.

- Ko HH, Hung CF, Wang JP, Lin CN. 2008. Antiinflammatory triterpenoids and steroids from *Ganoderma lucidum* and *G. tsugae*. Phytochemistry; **69**: 234-239.
- Kodama N, Komuta K, Nanba H. 2002. Can Maitake MD-fraction aid cancer patients. Alternative Medicine Review; **7**: 236-239.
- Korhonen K. 1978. "Interfertility and clonal size in the *Armillaria mellea* complex". Karstenia; **18**: 31-42.
- Lee SJ, Yeo WH, Yun S, Yoo D. 1999. Isolation and sequence analysis of new peptaibol, boletusin, from *Boletus* spp. Journal of Peptide Science; **5**: 374–378.
- Li X, Wang Z, Wang L, Walid E, Zhang H. 2012. *In Vitro* Antioxidant and Anti-Proliferation Activities of Polysaccharides from Various Extracts of Different Mushrooms. International Journal of Molecular Science; **13**: 5801-5817.
- Lin ES and Sung SC. 2006. Cultivating conditions influence exopolysaccharide production by the edible Basidiomycete *Antrodia cinnamomea* in submerged culture. International Journal of Food Microbiology; **108**: 182-187.
- Lin J, Nishino K, Roberts MC, Tolmasky M, Aminov RI, and Zhang L. 2015. Mechanisms of antibiotic resistance. Frontiers in Microbiology; **6**: 1-3.
- Martens UM, ed. 2010. "11 5-Azacytidine/Azacitidine". Small molecules in oncology. Recent Results in Cancer Research; **184**. Heidelberg: Springer: 159–170.
- Mattila P, Suonpaa K, Piironen V. 2000. Functional properties of edible mushrooms. Nutrition; **16**: 694-696.

- Mau JL, Tsai SY, Tseng YH, Huang SJ. 2005. Antioxidant properties of methanolic extracts from *Ganoderma tsugae*. Food Chemistry; **93**: 641-649.
- Mau JL, Tsai SY, Tseng YH, Huang SJ. 2005. Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. LWT; **38**: 589-597.
- Mayell M. 2001. Maitake extracts and their therapeutic potential. Alternative Medicine Review; **6**: 48-60.
- Mesaros NP, Nordmann P, Plesiat M, Roussel-Delvallez J, Van Eldere Y, Glupczynski Y, Van Laethem F, Jacobs P, Lebecque A, Malfroot PM, Tulkens and Van Bambeke F. 2007. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clinical Microbiology and Infection; **13**: 560–578.
- Mishra KK, Pal RS, Arunkumar R, Chandrashekara C, Jain SK, Bhatt JC. 2013. Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials. Food Chemistry; **138**: 1557-1563.
- Mlinaric A, Kac J, Pohleven F. 2005. Screening of selected wood-damaging fungi for the HIV-1 reverse transcriptase inhibitors. Acta Pharma; **55**: 69-79.
- Moonmoon M, Saleh A, Nasrat Jahan S, Asaduzzaman K. 2010. Cultivation of different strains of king oyster mushroom (*Pleurotus eryngii*) on saw dust and rice straw in Bangladesh. Saudi Journal of Biological Sciences; **17**: 341-345.
- Mori K, Kobayashi C, Tomita T, Inatomi S, Ikeda M. 2008. Antiatherosclerotic effect of the edible

- mushrooms *Pleurotus eryngii* (Eringi), *Grifola frondosa* (Maitake), and *Hypsizygus marmoreus* (Bunashimeji) in apolipoprotein E-deficient mice. *Nutrition Research*; **28**: 335-342.
- Morita K, Kobayashi S. 1967. Isolation, structure, and synthesis of lenthionine and its analogs. *Chemical Pharmaceutical Bulletin*; **15**: 988-93.
- Nakano H, Namatame K, Nemoto H, Motohashi H, Nishiyama K, Kumada K. 1999. A multi-institutional prospective study of lentinan in advanced gastric cancer patients with unrespectable and recurrent diseases: effect on prolongation of survival and improvement of quality of life. Kanagawa Lentinan Research Group. *Hepato-gastroenterology*; **46**: 2662-8.
- Nikaido, H. 2009. Multidrug Resistance in Bacteria. *Annual Review of Biochemistry*; **78**: 119-146.
- Nanba H, Kubo K. 1998. Antitumor substance extracted from *Grifola*. *U.S. Patent*; 5: 854,404.
- Oba K, Kobayashi M, Matsui T, Kodera Y, Sakamoto J. 2009. Individual patient based meta-analysis of lentinan for unresectable/recurrent gastric cancer. *Anticancer Research*; **29**: 2739-2745.
- Oduro KA, Munnecke DE, Sims JJ, Keen NT. 1976. Isolation of Antibiotics Produced in Culture by *Armillaria mellea*. *Transactions of British Mycological Society*; **66**: 195-199.
- Ozen T, Darcan C, Aktop O, Turkekul I. 2011. Screening of antioxidant, antimicrobial activities

- and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey. Combinatorial Chemistry & High Throughput Screening; **14**: 72-84.
- Ozturk M, Duru ME, Kivrak S, Mercan-Dogan N, Turkoglu A, Ozler MA. 2011. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms. Food Chemical Toxicology; **49**: 1353-1360.
- Pankey GA and Sabath LD. 2004. Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections. Clinical Infectious Diseases; **38**: 864–870.
- Poucheret P, Fons F, Rapior S. 2006. Biological and Pharmacological Activity of Higher Fungi: 20-Year Retrospective Analysis. Mycologie; **27**: 311-333.
- Quereshi S, Pandey AK, Sandhu SS. 2010. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. Journal of Scientific Research; **3**: 9-13.
- Rodriguez-Ortiz R, Limon MC, Avalos J. 2009. Regulation of carotenogenesis and secondary metabolism by nitrogen in wild-type *Fusarium fujikuroi* and carotenoid-overproducing mutants. Applications of Environmental Microbiology; **75**: 405-413.
- Sanchez S, Chavez A, Forero A, Garcia-Huante Y, Romero A, Sanchez M, Rocha D, Sanchez B, Avalos M, Guzmán-Trampe S, Rodríguez-Sanoja R, Langley E, Ruiz B. 2010. Carbon source regulation of antibiotic production. The Journal of Antibiotics; **63**: 442-459.

Sanglard D. 2002. Clinical relevance of mechanisms of antifungal drug resistance in yeasts.

Enferm Infecc Microbiol Clin; **20**: 462-469.

Sarangi I, Ghosh D, Kumar Bhutia S, Kumar Mallick S, Maiti TK. 2006. Anti-tumor and immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans.

International Immunopharmacology; **6**: 1287-1297.

Schulz B, Boyle C, Siegfried D, Römmert AK and Karsten K. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research; **106**: 96-1004.

Sharma AK, Dr. A M Jana, Dr. Archana Srivastav, Dr. Madhu Gupta, Sachin Sharma, Sher Singh Gill. 2014. Antimicrobial Properties of Some Edible Mushrooms: A Review. World Journal of Pharmacy and Pharmaceutical Sciences; **3**: 1009-1023.

Shaw CG, Glen A K. 1991. Armillaria Root Disease. Agriculture Handbook No. 691. Washington, D.C.

Sinanoglou JV, Zoumpoulakis P, Heropoulos G, Proestos C, Ćirić A, Petrovic J, Glamoclija J, Sokovic M. 2015. Lipid and fatty acid profile of the edible fungus *Laetiporus sulphureus*. Antifungal and antibacterial properties. Journal of Food Science and Technology; **52**: 3264-3272.

Smith ML, Duchesne LC, Bruhn JN and Anderson JB. 1990. Mitochondrial Genetics in a Natural Population of the Plant Pathogen *Armillaria*. Genetics; **126**: 575-582.

Sulavik MC, Gambino LF, Miller PF. 1995. The *MarR* Repressor of the Multiple Antibiotic Resistance (*mar*) Operon *Escherichia coli*: Prototypic Member of a Family of Bacterial

- Regulatory Proteins Involved in Sensing Phenolic Compounds. *Molecular Medicine*; **1**: 436-446.
- The State Pharmacopoeia Commission of P. R. China. State Pharmacopoeia Commission of the People's Republic of China. Beijing, China: Chemical Industry Press, 2000.
- Tochikura T, Nakashima H, Ohashi Y, Yamamoto N. 1998. Inhibition (in vitro) of replication and of the cytopathic effect of human immunodeficiency virus by an extract of the culture medium of *Lentinus edodes* mycelia. *Medical Microbiology and Immunology*; **177**: 235-244.
- Tong H, Xia F, Feng K, Sun G, Gao X, Sun L, Jiang R, Tian D, Sun X. 2009. Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. *Bioresource Technology*; **100**: 1682–1686.
- Tsai SY, Tsai HL, Mau JL. 2007. Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindracea*, and *Boletus edulis*. *LWT*; **40**: 1392–1402.
- Tseng YH, Yang JH, Mau JL. 2008. Antioxidant properties of polysaccharides from *Ganoderma tsugae*. *Food Chemistry*; **107**: 732-738.
- Turkoglu A, Duru ME, Mercan N, Kivra I, Gezer K. 2007. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*; **101**: 267-273.

- Uzun Y, Atalan E, Keles A, Demirel K. 2004. *Pleurotus eryngii* (DC. ex Fr.) Quel. ve *Agrocybe cylindracea* (DC. Fr.) Maire makrofunguslarının antimikrobiyal aktivitesi. Mimar Sinan Güzel Sanatlar Üniversitesi Fen Edebiyat Fakültesi Dergisi 4, 125-133.
- Vamanu E, Sultana N. 2013. Antioxidant Capacity and the Correlation with Major Phenolic Compounds, Anthocyanin, and Tocopherol Content in Various Extracts from the Wild Edible *Boletus edulis* Mushroom. BioMedical Research International; 2013, Article ID 313905, 11 pages.
- Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF. 2011. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Chapter 9: *Ganoderma lucidum* (Lingzhi or Reishi) A Medicinal Mushroom.
- Walkowiak S, Subramaniam R. 2014. A nitrogen-responsive gene affects virulence in *Fusarium graminearum*. Canadian Journal of Plant Pathology; **36**: 224-234.
- Wang H, Ng TB. 2006. Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. Peptides; **27**: 27-30.
- Wegener HC. 1999. The Consequences for Food Safety of the Use of Fluoroquinolones in Food Animals. The New England Journal of Medicine Editorial; **340**: 1580-1582.
- Wiegand I, Kai H, Hancock REW. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols; **3**: 163-175.
- Wierzbowski AK, Nichol K, Laing N, Hisanaga T, Nikulin A, Karlowsky JA, Hoban DJ, and Zhanel

- GG. 2007. Macrolide resistance mechanisms among *Streptococcus pneumoniae* isolated over 6 years of Canadian Respiratory Organism Susceptibility Study. Journal of Antimicrobial Chemotherapy; **60**: 733-740.
- Williams RB, Henrikson JC, Hoover AR, Lee AE, Cichewicz RH. 2008. Epigenetic remodeling of the fungal secondary metabolome. Organic and Biomolecular Chemistry; **6**: 1895-1897.
- Wong A and Seguin K. 2015. Effects of genotype on rates of substitution during experimental evolution. International Journal of Organic Evolution; **69**: 1772-1785.
- Xu D, Sheng Y, Zhou ZY, Liu R, Leng Y, Liu JK. 2009. Sesquiterpenes from cultures of the basidiomycete *Clitocybe conglobata* and their 11 β -hydroxysteroid dehydrogenase inhibitory activity. Chemical and Pharmaceutical Bulletin; **57**: 433-435.
- Yasumoto K, Iwami K, Mitsuda H. 1971. A new sulphur-containing peptide from *Lentinus edodes* acting as precursor for lenthionine. Agricultural and Biological Chemistry; **35**: 2059-69.
- Ying J, Mao X, Ma Q, Zong Y, Wen H. 1987. Icones of medicinal fungi from China. Science Press, Beijing, Chine, 575.
- Yang JH, Lin HC, Mau JL. 2002. Antioxidant properties of several commercial mushrooms. Food Chemistry; **77**: 229-235.
- Yang Z, Xu J, Fu Q, Fu X, Shu T, Bi Y, Song B. 2013. Antitumor activity of a polysaccharide from *Pleurotus eryngii* on mice bearing renal cancer. Carbohydrate Polymers; **95**: 615-620.
- Yang-Hao Y, Han-Peng K, Hui-Hsia H, Jhy-Wei L, Wu-Huei H, Shih-Jung C, Muh-Hwan S, Shwu-

Huey L, Yung-Chi C, Chih-Yi C, and Ming-Ching K. 2012. *Ganoderma tsugae* Induces S Phase Arrest and Apoptosis in Doxorubicin-Resistant Lung Adenocarcinoma H23/0.3 Cells via Modulation of the PI3K/Akt Signaling Pathway. *Evidence-Based Complementary and Alternative Medicine*; **2012**, Article ID 371286, 13 pages.

Zhang D, Frankowska A, Jarzy ska G, Kojta AK, Drewnowskam M, Wydmaska D, Bielawski L, Wang J, Falandysz J. 2010. Metals of King Bolete (*Boletus edulis*) Bull.: Fr. collected at the same site over two years. *African Journal of Agricultural Research*; **5**: 3050-3055.

Appendix I- Plate Outlines

i) Outline of MIC tests performed on *E. coli* strains, *marR* R77H, *gyrA* S83L and wild-type MG1655 susceptibility to fungal crude extracts. Wells contained 50µL of LB medium with 50µL fungal crude extract (CE) serially diluted across the plate as shown above and 150µL of *E. coli* suspension. Blanks consisted of fungal CE without *E. coli* suspension, reference wells contained the LB medium and *E. coli* suspension only, and medium control contained LB medium only.

ii) Outline of MIC tests performed on *S. cerevisiae* S288C susceptibility to fungal crude extracts. Well's contained 50 μ L of YPD medium with 50 μ L fungal crude extract (CE) serially diluted across the plate as shown above and 150 μ L of *S. cerevisiae* suspension. Blanks consisted of fungal CE and YPD without *S. cerevisiae* suspension, reference wells contained the YPD medium and *S. cerevisiae* suspension only, and medium control contained only YPD medium.

Appendix II-MIC Results Summaries

i) MIC50, MIC90, MIC100 values for *Lentinula edodes* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, SAC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC 100 (range) mycelia extract	MIC 100 (range) media extract
LN						
<i>marR</i>	2.8 < MIC ≤ 5.6 1.4 < MIC ≤ 2.8	3.4 < MIC ≤ 6.9 1.72 < MIC ≤ 3.43	MIC > 11.3 MIC > 11.3	MIC > 13.8 MIC > 13.8	MIC > 11.3 MIC > 11.3	MIC > 13.8 MIC > 13.8
<i>gyrA</i>	2.8 < MIC ≤ 5.6 1.4 < MIC ≤ 2.8	3.4 < MIC ≤ 6.9 1.72 < MIC ≤ 3.43	MIC > 11.3 MIC > 11.3	MIC > 13.8 MIC > 13.8	MIC > 11.3 MIC > 11.3	MIC > 13.8 MIC > 13.8
MG1655	1.4 < MIC ≤ 2.8 0.7 < MIC ≤ 1.4	3.4 < MIC ≤ 6.9 1.72 < MIC ≤ 3.43	MIC > 11.3 MIC > 11.3	MIC > 13.8 MIC > 13.8	MIC > 11.3 MIC > 11.3	MIC > 13.8 MIC > 13.8
S288C	5.6 < MIC ≤ 11.3	MIC > 13.8	MIC > 11.3	MIC > 13.8	MIC > 11.3	MIC > 13.8
EC						
<i>marR</i>	2.4 < MIC ≤ 4.8 2.4 < MIC ≤ 4.8	ND 0.99 < MIC ≤ 1.98	4.8 < MIC ≤ 9.5 MIC > 9.5	7.9 < MIC ≤ 15.8 15.8 < MIC < 31.8	MIC > 9.5 MIC > 9.5	15.8 < MIC ≤ 31.8 15.8 < MIC ≤ 31.8
<i>gyrA</i>	ND 2.4 < MIC ≤ 4.8	ND 0.99 < MIC ≤ 1.98	4.8 < MIC ≤ 9.5 MIC > 9.5	7.9 < MIC ≤ 15.8 15.8 < MIC < 31.8	MIC > 9.5 MIC > 9.5	15.8 < MIC ≤ 31.8 15.8 < MIC ≤ 31.8
MG1655	0.6 < MIC ≤ 1.19 2.4 < MIC ≤ 4.8	ND 0.99 < MIC ≤ 1.98	4.8 < MIC ≤ 9.5 MIC > 9.5	7.9 < MIC ≤ 15.8 15.8 < MIC < 31.8	MIC > 9.5 MIC > 9.5	15.8 < MIC ≤ 31.8 15.8 < MIC ≤ 31.8
S288C	4.75 < MIC ≤ 9.5	2.75 < MIC < 5.5	MIC > 9.5	2.75 < MIC < 5.5	MIC > 9.5	2.75 < MIC ≤ 5.5
SAC						
<i>marR</i>	2.4 < MIC ≤ 4.8 2.4 < MIC ≤ 4.8	2.14 < MIC ≤ 4.28 1.07 < MIC ≤ 2.14	MIC > 9.5 MIC > 9.5	17.1 < MIC < 34.3 ND	MIC > 9.5 MIC > 9.5	17.1 < MIC ≤ 34.3 17.1 < MIC ≤ 34.3
<i>gyrA</i>	2.4 < MIC ≤ 4.8 2.4 < MIC ≤ 4.8	2.14 < MIC ≤ 4.28 1.07 < MIC ≤ 2.14	MIC > 9.5 MIC > 9.5	17.1 < MIC < 34.4 ND	MIC > 9.5 MIC > 9.5	17.1 < MIC ≤ 34.3 17.1 < MIC ≤ 34.3
MG1655	2.4 < MIC ≤ 4.8 2.4 < MIC ≤ 4.8	ND 1.07 < MIC ≤ 2.14	MIC > 9.5 MIC > 9.5	ND ND	MIC > 9.5 MIC > 9.5	17.1 < MIC ≤ 34.3 15.8 < MIC ≤ 31.8
S288C	MIC > 9.5	17.1 < MIC < 34.4	MIC > 9.5	17.1 < MIC < 34.3	MIC > 9.5	17.1 < MIC ≤ 34.3
UI						
<i>marR</i>	3.75 < MIC ≤ 7.5 ND	3.2 < MIC ≤ 6.3 0.8 < MIC ≤ 1.6	MIC > 15 ND	MIC > 25.25 12.6 < MIC ≤ 25.3	MIC > 15 ND	MIC > 25.3 MIC > 25.3
<i>gyrA</i>	3.75 < MIC ≤ 7.5 3.75 < MIC ≤ 7.5	1.6 < MIC ≤ 3.2 0.8 < MIC ≤ 1.6	MIC > 15 MIC > 15	12.6 < MIC ≤ 25.3 MIC > 25.25	MIC > 15 MIC > 15	MIC > 25.3 MIC > 25.3
MG1655	3.75 < MIC ≤ 7.5 3.75 < MIC ≤ 7.5	0.8 < MIC ≤ 1.6 0.8 < MIC ≤ 1.6	MIC > 15 MIC > 15	12.6 < MIC ≤ 25.3 MIC > 25.25	MIC > 15 MIC > 15	MIC > 25.3 MIC > 25.3
S288C	MIC > 15	12.6 < MIC < 25.3	MIC > 15	12.6 < MIC < 25.3	MIC > 15	12.6 < MIC ≤ 25.3

ii) MIC50, MIC90, MIC100 values for *Pleurotus ostreatus* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC 100 (range) mycelia extract	MIC 100 (range) media extract
LN						
<i>marR</i>	$5.06 < MIC \leq 10.12$	$1.94 < MIC \leq 3.88$	$40.5 < MIC \leq 81$	$7.75 < MIC \leq 15.5$	$MIC > 81$	$MIC > 15.5$
<i>gyrA</i>	$40.5 < MIC < 81$	$1.94 < MIC \leq 3.88$	$40.5 < MIC \leq 81$	$7.75 < MIC \leq 15.5$	$MIC > 81$	$MIC > 15.5$
MG1655	$40.5 < MIC < 81$	$3.85 < MIC \leq 7.75$	$40.5 < MIC \leq 81$	$7.75 < MIC \leq 15.5$	$MIC > 81$	$MIC > 15.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	$2.53 < MIC \leq 5.06$	$3.88 < MIC \leq 7.75$	$MIC > 20.25$	$MIC > 15.5$	$MIC > 20.25$	$MIC > 15.5$
<i>gyrA</i>	$2.53 < MIC \leq 5.06$	$7.75 < MIC < 15.5$	$MIC > 20.25$	$MIC > 15.5$	$MIC > 20.25$	$MIC > 15.5$
MG1655	$2.53 < MIC \leq 5.06$	$1.94 < MIC \leq 3.88$	$MIC > 20.25$	$MIC > 15.5$	$MIC > 20.25$	$MIC > 15.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
5AC						
<i>marR</i>	$0.3 < MIC \leq 0.6$	$5.6 < MIC \leq 11.25$	$MIC > 9.5$	$MIC > 22.5$	$MIC > 9.5$	$MIC > 22.5$
<i>gyrA</i>	$0.6 < MIC \leq 1.19$	$11.25 < MIC < 22.5$	$MIC > 9.5$	$MIC > 22.5$	$MIC > 9.5$	$MIC > 22.5$
MG1655	$1.19 < MIC \leq 2.38$	$11.25 < MIC < 22.5$	$MIC > 9.5$	$MIC > 22.5$	$MIC > 9.5$	$MIC > 22.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	$3.56 < MIC \leq 7.13$	$0.45 < MIC \leq 0.89$	$MIC > 28.5$	$MIC > 28.5$	$MIC > 28.5$	$MIC > 28.5$
<i>gyrA</i>	$3.56 < MIC \leq 7.13$	$0.89 < MIC \leq 1.78$	$MIC > 28.5$	$MIC > 28.5$	$MIC > 28.5$	$MIC > 28.5$
MG1655	$1.78 < MIC \leq 3.56$	$0.22 < MIC \leq 0.45$	$MIC > 28.5$	$MIC > 28.5$	$MIC > 28.5$	$MIC > 28.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A

iii) MIC50, MIC90, MIC100 values for *Pleurotus eryngii* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, SAC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC50 (range) mycelia extract	MIC50 (range) media extract	MIC90 (range) mycelia extract	MIC90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	0.44 < MIC ≤ 0.88 1.75 < MIC ≤ 3.5	1.38 < MIC ≤ 2.75 0.68 < MIC ≤ 1.38	1.75 < MIC ≤ 3.5 MIC > 14	MIC > 5.5 MIC > 5.5	MIC > 3.5 MIC > 14	MIC > 5.5 MIC > 5.5
<i>gyrA</i>	0.88 < MIC ≤ 1.75 1.75 < MIC ≤ 3.5	2.75 < MIC ≤ 5.5 0.68 < MIC ≤ 1.38	MIC > 3.5 MIC > 14	MIC > 5.5 MIC > 5.5	MIC > 3.5 MIC > 14	MIC > 5.5 MIC > 5.5
MG1655	0.88 < MIC ≤ 1.75 1.75 < MIC ≤ 3.5	0.68 < MIC ≤ 1.38 0.68 < MIC ≤ 1.38	MIC > 3.5 MIC > 14	MIC > 5.5 MIC > 5.5	MIC > 3.5 MIC > 14	MIC > 5.5 MIC > 5.5
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	0.22 < MIC ≤ 0.44 0.22 < MIC ≤ 0.44	0.44 < MIC ≤ 0.88 0.11 < MIC ≤ 0.22	0.88 < MIC ≤ 1.75 MIC > 7	MIC > 7 MIC > 7	MIC > 1.75 MIC > 7	MIC > 7 MIC > 7
<i>gyrA</i>	0.44 < MIC ≤ 0.88 0.22 < MIC ≤ 0.44	0.88 < MIC ≤ 1.75 0.22 < MIC ≤ 0.44	0.875 < MIC ≤ 1.75 MIC > 7	MIC > 7 MIC > 7	MIC > 1.75 MIC > 7	MIC > 7 MIC > 7
MG1655	0.22 < MIC ≤ 0.44 0.44 < MIC ≤ 0.88	0.22 < MIC ≤ 0.44 0.11 < MIC ≤ 0.22	0.44 < MIC ≤ 0.88 MIC > 7	MIC > 7 MIC > 7	MIC > 1.75 MIC > 7	MIC > 7 MIC > 7
S288C	N/A	N/A	N/A	N/A	N/A	N/A
SAC						
<i>marR</i>	0.59 < MIC ≤ 1.19 1.17 < MIC ≤ 2.35	0.78 < MIC ≤ 0.39 0.48 < MIC ≤ 0.85	0.39 < MIC ≤ 0.78 MIC > 18.75	MIC > 6.25 MIC > 6.25	MIC > 4.75 MIC > 18.75	MIC > 6.25 MIC > 6.25
<i>gyrA</i>	1.19 < MIC ≤ 2.38 1.17 < MIC ≤ 2.35	0.78 < MIC ≤ 1.56 0.2 < MIC ≤ 0.4	MIC > 4.75 MIC > 18.8	MIC > 6.25 MIC > 6.25	MIC > 4.75 MIC > 18.75	MIC > 6.25 MIC > 6.25
MG1655	0.59 < MIC ≤ 1.19 2.34 < MIC ≤ 4.69	0.19 < MIC ≤ 0.39 0.2 < MIC ≤ 0.4	2.37 < MIC ≤ 4.75 MIC > 18.8	MIC > 6.25 MIC > 6.25	MIC > 4.75 MIC > 18.75	MIC > 6.25 MIC > 6.25
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	2.75 < MIC ≤ 5.5 1.17 < MIC ≤ 2.34	0.39 < MIC ≤ 0.78 0.1 < MIC ≤ 0.2	MIC > 5.5 11 < MIC ≤ 22	MIC > 6.25 MIC > 6.25	MIC > 5.5 MIC > 22	MIC > 6.25 MIC > 6.25
<i>gyrA</i>	1.38 < MIC ≤ 2.75 2.75 < MIC ≤ 5.5	0.78 < MIC ≤ 1.56 0.2 < MIC ≤ 0.4	MIC > 5.5 11 < MIC ≤ 22	MIC > 6.25 MIC > 6.25	MIC > 5.5 MIC > 22	MIC > 6.25 MIC > 6.25
MG1655	0.69 < MIC ≤ 1.38 2.75 < MIC ≤ 5.5	0.39 < MIC ≤ 0.78 0.1 < MIC ≤ 0.2	MIC > 5.5 11 < MIC ≤ 22	MIC > 6.25 MIC > 6.25	MIC > 5.5 MIC > 22	MIC > 6.25 MIC > 6.25
S288C	N/A	N/A	N/A	N/A	N/A	N/A

ix) MIC50, MIC90, MIC100 values for *Laetiporus sulphureus* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC 100 (range) mycelia extract	MIC 100 (range) media extract
LN						
<i>marR</i>	MIC > 20.8 10.4 < MIC ≤ 20.8	0.7 < MIC ≤ 1.35 1.5 < MIC ≤ 1.65	MIC > 20.8 MIC > 20.8	5.43 < MIC ≤ 10.9 MIC > 21.8	MIC > 20.8 MIC > 20.8	10.9 < MIC ≤ 21.8 MIC > 21.8
<i>gyrA</i>	MIC > 20.8 5.2 < MIC ≤ 10.4	1.5 < MIC ≤ 1.65 2.72 < MIC ≤ 5.43	MIC > 20.8 10.4 < MIC ≤ 20.8	5.43 < MIC ≤ 10.9 MIC > 21.8	MIC > 20.8 MIC > 20.8	10.9 < MIC ≤ 21.8 MIC > 21.8
MG1655	ND 10.4 < MIC ≤ 20.8	0.34 < MIC ≤ 0.68 5.2 < MIC ≤ 10.4	ND MIC > 20.8	0.68 < MIC ≤ 1.36 MIC > 21.8	ND MIC > 20.8	1.36 < MIC ≤ 2.71 MIC > 21.8
S288C	0.65 < MIC ≤ 1.3 0.08 < MIC ≤ 1.6	21.8 < MIC < 10.9 2.7 < MIC ≤ 5.4	1.3 < MIC ≤ 2.6 0.16 < MIC ≤ 2.32	10.9 < MIC ≤ 21.8 2.7 < MIC ≤ 5.4	MIC > 20.8 2.6 < MIC ≤ 5.2	MIC > 21.8 5.4 < MIC ≤ 10.9
EC						
<i>marR</i>	6.62 < MIC ≤ 13.3 MIC > 13.3	11.6 < MIC ≤ 23.3 2.9 < MIC ≤ 5.8	MIC > 13.3 MIC > 13.3	MIC > 46.5 11.6 < MIC ≤ 23.3	MIC > 13.3 MIC > 13.3	MIC > 46.5 23.3 < MIC ≤ 46.5
<i>gyrA</i>	MIC > 13.3 6.62 < MIC ≤ 13.3	5.8 < MIC ≤ 11.6 1.45 < MIC ≤ 2.9	MIC > 13.3 MIC > 13.3	MIC > 46.5 11.6 < MIC ≤ 23.3	MIC > 13.3 MIC > 13.3	MIC > 46.5 23.3 < MIC ≤ 46.5
MG1655	ND 6.62 < MIC ≤ 13.3	5.8 < MIC ≤ 11.6 1.45 < MIC ≤ 2.9	ND MIC > 13.3	6.62 < MIC ≤ 13.3 11.6 < MIC ≤ 23.3	ND MIC > 13.3	MIC > 46.5 23.3 < MIC ≤ 46.5
S288C	MIC > 13.3 MIC > 13.3	6.63 < MIC < 13.3 2.9 < MIC ≤ 5.8	MIC > 13.3 MIC > 13.3	6.63 < MIC < 13.3 5.8 < MIC < 11.6	MIC > 13.3 MIC > 13.3	11.6 < MIC ≤ 23.3 5.8 < MIC ≤ 11.6
5AC						
<i>marR</i>	4.75 < MIC ≤ 9.5 2.37 < mic ≤ 4.75	1.7 < MIC ≤ 3.4 0.85 < MIC ≤ 1.7	MIC > 9.5 MIC > 9.5	6.8 < MIC ≤ 13.6 6.8 < MIC ≤ 13.6	MIC > 9.5 MIC > 9.5	MIC > 27.3 13.6 < MIC ≤ 27.3
<i>gyrA</i>	4.75 < MIC ≤ 9.5 4.75 < MIC ≤ 9.5	1.7 < MIC ≤ 3.4 0.85 < MIC ≤ 1.7	MIC > 9.5 MIC > 9.5	13.6 < MIC ≤ 27.3 6.8 < MIC ≤ 13.6	MIC > 9.5 MIC > 9.5	MIC > 27.3 13.6 < MIC ≤ 27.3
MG1655	ND 2.37 < MIC ≤ 4.75	1.7 < MIC ≤ 3.4 0.85 < MIC ≤ 1.7	ND MIC > 9.5	3.4 < MIC ≤ 6.81 6.8 < MIC ≤ 13.6	ND MIC > 9.5	MIC > 27.3 13.6 < MIC ≤ 27.3
S288C	4.75 < MIC ≤ 9.5	6.8 < MIC ≤ 13.6	MIC > 9.5	13.6 < MIC < 27.3	MIC > 9.5	13.6 < MIC ≤ 27.3
UI						
<i>marR</i>	0.88 < MIC ≤ 1.75 0.44 < MIC ≤ 0.88	1.8 < MIC ≤ 3.6 0.9 < MIC ≤ 1.8	3.5 < MIC ≤ 7 3.5 < MIC ≤ 7	7.2 < MIC ≤ 14.4 7.2 < MIC ≤ 14.4	MIC > 7 MIC > 7	14.4 < MIC ≤ 28.8 14.4 < MIC ≤ 28.8
<i>gyrA</i>	0.88 < MIC ≤ 1.75 1.75 < MIC ≤ 3.5	1.8 < MIC ≤ 3.6 0.9 < MIC ≤ 1.8	1.75 < MIC ≤ 3.5 3.5 < MIC ≤ 7	7.2 < MIC ≤ 14.4 7.2 < MIC ≤ 14.4	MIC > 7 MIC > 7	14.4 < MIC ≤ 28.8 14.4 < MIC ≤ 28.8
MG1655	ND 1.75 < MIC ≤ 3.5	0.22 < MIC ≤ 0.44 0.45 < MIC ≤ 0.9	ND MIC > 7	3.9 < MIC ≤ 7.2 3.9 < MIC ≤ 7.2	ND MIC > 7	7.2 < MIC ≤ 14.4 7.2 < MIC ≤ 14.4
S288C	ND	14.4 < MIC < 28.8	ND	14.4 < MIC ≤ 28.8	ND	MIC > 28.8

x) MIC50, MIC90, MIC100 values for *Agaricus bisporus* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC 100 (range) mycelia extract	MIC 100 (range) media extract
LN						
<i>marR</i>	MIC > 9.5	MIC > 10.25	MIC > 9.5	MIC > 10.25	MIC > 9.5	MIC > 10.25
<i>gyrA</i>	$4.75 < MIC \leq 9.5$	MIC > 10.25	MIC > 9.5	MIC > 10.25	MIC > 9.5	MIC > 10.25
MG1655	$2.4 < MIC \leq 4.75$	$5.1 < MIC \leq 10.25$	MIC > 9.5	MIC > 10.25	MIC > 9.5	MIC > 10.25
S288C	MIC > 9.5	MIC > 10.25	MIC > 9.5	MIC > 10.25	MIC > 9.5	MIC > 10.25
EC						
<i>marR</i>	$3.1 < MIC \leq 6.25$	$1.8 < MIC \leq 3.6$	MIC > 6.25	$14.25 < MIC \leq 28.5$	MIC > 6.25	MIC > 28.5
<i>gyrA</i>	$3.1 < MIC \leq 6.25$	$1.8 < MIC \leq 3.6$	MIC > 6.25	$14.25 < MIC \leq 28.5$	MIC > 6.25	MIC > 28.5
MG1655	$3.1 < MIC \leq 6.25$	$0.9 < MIC \leq 1.8$	MIC > 6.25	$14.25 < MIC \leq 28.5$	MIC > 6.25	MIC > 28.5
S288C	MIC > 6.25	MIC > 28.5	MIC > 6.25	MIC > 28.5	MIC > 6.25	MIC > 28.5
5AC						
<i>marR</i>	$7.1 < MIC \leq 14.25$	$4.6 < MIC \leq 9.2$	MIC > 14.25	$18.4 < MIC \leq 36.8$	MIC > 14.25	MIC > 36.8
<i>gyrA</i>	$7.1 < MIC \leq 14.25$	$2.3 < MIC \leq 4.6$	MIC > 14.25	$18.4 < MIC \leq 36.8$	MIC > 14.25	MIC > 36.8
MG1655	$3.6 < MIC \leq 7.1$	$1.2 < MIC \leq 2.3$	MIC > 14.25	$18.4 < MIC \leq 36.8$	MIC > 14.25	MIC < 36.8
S288C	MIC > 14.25	MIC > 36.8	MIC > 14.25	MIC > 36.8	MIC > 14.25	MIC > 36.8
UI						
<i>marR</i>	$20 < MIC \leq 40$	$4.3 < MIC \leq 8.6$	MIC > 40	MIC > 34.25	MIC > 40	MIC > 34.25
<i>gyrA</i>	$10 < MIC \leq 20$	$2.1 < MIC \leq 4.3$	MIC ≥ 40	MIC > 34.25	MIC > 40	MIC > 34.25
MG1655	$10 < MIC \leq 20$	$2.1 < MIC \leq 4.3$	MIC ≥ 40	MIC > 34.25	MIC > 40	MIC > 34.25
S288C	MIC > 40	MIC > 34.25	MIC > 40	MIC > 34.25	MIC > 40	MIC > 34.25

xi) MIC50, MIC90, MIC100 values for *Armillaria mellea* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, SAC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	$1.47 < MIC \leq 2.94$	$1.94 < MIC \leq 2.88$	$MIC > 23.5$	$MIC > 15.5$	$MIC > 23.5$	$MIC > 15.5$
<i>marR</i>	$2.94 < MIC \leq 5.88$	$1.94 < MIC \leq 2.88$	$MIC > 23.5$	$MIC > 15.5$	$MIC > 23.5$	$MIC > 15.5$
MG1655	$2.94 < MIC \leq 5.88$	$3.88 < MIC \leq 7.75$	$MIC > 23.5$	$MIC > 15.5$	$MIC > 23.5$	$MIC > 15.5$
S288C	$MIC > 4$ $MIC > 23.5$	$MIC > 15$ $MIC > 15.5$	$MIC > 4$ $MIC > 23.5$	$MIC > 15$ $MIC > 15.5$	$MIC > 4$ $MIC > 23.5$	$MIC > 15$ $MIC > 15.5$
EC						
<i>marR</i>	$0.78 < MIC \leq 1.56$	$0.94 < MIC \leq 1.88$	$MIC > 25$	$15 < MIC \leq 30$	$MIC > 25$	$MIC > 60$
<i>gyrA</i>	$0.78 < MIC \leq 1.56$	$0.94 < MIC \leq 1.88$	$MIC > 25$	$30 < MIC \leq 60$	$MIC > 25$	$MIC > 60$
MG1655	$1.56 < MIC \leq 3.13$	$1.88 < MIC \leq 3.75$	$MIC > 25$	$MIC > 60$	$MIC > 25$	$MIC > 60$
S288C	$MIC > 39.3$ $MIC > 25$	$MIC > 49.5$ $MIC > 60$	$MIC > 39.3$ $MIC > 25$	$MIC > 49.5$ $MIC > 60$	$MIC > 39.3$ $MIC > 25$	$MIC > 49.5$ $MIC > 60$
SAC						
<i>marR</i>	$0.39 < MIC \leq 0.78$	$0.51 < MIC \leq 1.02$	$12.5 < MIC \leq 25$	$32.5 < MIC \leq 65$	$MIC > 25$	$MIC > 65$
<i>gyrA</i>	$0.78 < MIC \leq 1.56$	$1.02 < MIC \leq 2.03$	$12.5 < MIC \leq 25$	$32.5 < MIC \leq 65$	$MIC > 25$	$MIC > 65$
MG1655	$1.56 < MIC \leq 3.13$	$1.02 < MIC \leq 2.03$	$MIC > 25$	$MIC > 65$	$MIC > 25$	$MIC > 65$
S288C	$MIC > 18.8$ $MIC > 25$	$MIC > 37.5$ $MIC > 60$	$MIC > 18.8$ $MIC > 25$	$MIC > 37.5$ $MIC > 60$	$MIC > 18.8$ $MIC > 25$	$MIC > 37.5$ $MIC > 60$
UI						
<i>marR</i>	$0.78 < MIC \leq 1.56$	$0.39 < MIC \leq 0.78$	$12.5 < MIC \leq 25$	$25 < MIC \leq 50$	$MIC > 25$	$MIC > 50$
<i>gyrA</i>	$0.78 < MIC \leq 1.56$	$0.78 < MIC \leq 1.56$	$12.5 < MIC \leq 25$	$MIC > 50$	$MIC > 25$	$MIC > 50$
MG1655	$3.13 < MIC \leq 0.78$	$1.56 < MIC \leq 0.78$	$12.5 < MIC \leq 25$	$25 < MIC \leq 50$	$MIC > 25$	$MIC > 50$
S288C	$MIC > 39.3$ $MIC > 25$	$MIC > 24.3$ $MIC > 50$	$MIC > 39.3$ $MIC > 25$	$MIC > 24.3$ $MIC > 50$	$MIC > 39.3$ $MIC > 25$	$MIC > 24.3$ $MIC > 50$

xii) MIC50, MIC90, MIC100 values for *Armillaria solidipes* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC 100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	MIC > 9.5	2.9 < MIC ≤ 5.8 2.9 < MIC ≤ 5.8	MIC > 9.5	MIC > 23.3 MIC > 23.3	MIC > 9.5	MIC > 23.3 MIC > 23.3
<i>gyrA</i>	4.75 < MIC ≤ 9.5	2.9 < MIC ≤ 5.8 2.9 < MIC ≤ 5.8	MIC > 9.5	MIC > 23.3 MIC > 23.3	MIC > 9.5	MIC > 23.3 MIC > 23.3
MG1655	2.38 < MIC ≤ 9.5	2.9 < MIC ≤ 5.8 2.9 < MIC ≤ 5.8	MIC > 9.5	MIC > 23.3 MIC > 23.3	MIC > 9.5	MIC > 23.3 MIC > 23.3
S288C	MIC > 6.5	MIC > 40.3	MIC > 6.5	MIC > 40.3	MIC > 6.5	MIC > 40.3
EC						
<i>marR</i>	1.75 < MIC ≤ 3.5	0.65 < MIC ≤ 1.3 0.65 < MIC ≤ 1.3	MIC > 7	MIC > 21 21 < MIC ≤ 10.5	MIC > 7	MIC > 21 MIC > 21
<i>gyrA</i>	1.75 < MIC ≤ 3.5	0.65 < MIC ≤ 1.3 0.33 < MIC ≤ 0.65	MIC > 7	MIC > 21 MIC > 21	MIC > 7 N/A	MIC > 21 MIC > 21
MG1655	1.75 < MIC ≤ 3.5	0.65 < MIC ≤ 1.3 0.65 < MIC ≤ 1.3	MIC > 7	MIC > 21 MIC > 21	MIC > 7	MIC > 21 MIC > 21
S288C	MIC > 7	MIC > 21	MIC > 7	MIC > 21	MIC > 7	MIC > 21
5AC						
<i>marR</i>	3.09 < MIC ≤ 6.19	0.79 < MIC ≤ 1.58 0.79 < MIC ≤ 1.58	6.19 < MIC ≤ 12.4	MIC > 25.3 MIC > 25.3	12.4 < MIC ≤ 24.8	MIC > 25.3 MIC > 25.3
<i>gyrA</i>	3.09 < MIC ≤ 6.19	0.39 < MIC ≤ 0.79 0.39 < MIC ≤ 0.79	6.19 < MIC ≤ 12.4	MIC > 25.3 MIC > 25.3	12.4 < MIC ≤ 24.8	MIC > 25.3 MIC > 25.3
MG1655	6.19 < MIC ≤ 12.4	0.79 < MIC ≤ 1.58 0.79 < MIC ≤ 1.58	6.19 < MIC ≤ 12.4	MIC > 25.3 MIC > 25.3	12.4 < MIC ≤ 24.8	MIC > 25.3 MIC > 25.3
S288C	MIC > 49.5	MIC > 25.25	MIC > 49.5	MIC > 25.3	MIC > 49.5	MIC > 25.3
UI						
<i>marR</i>	8.53 < MIC ≤ 17.1	0.89 < MIC ≤ 1.78 0.89 < MIC ≤ 1.78	MIC > 68.3	MIC > 28.5 MIC > 28.5	MIC > 68.3	MIC > 28.5 MIC > 28.5
<i>gyrA</i>	17.1 < MIC ≤ 34.1	0.89 < MIC ≤ 1.78 0.89 < MIC ≤ 1.78	MIC > 68.3	MIC > 28.5 MIC > 28.5	MIC > 68.3	MIC > 28.5 MIC > 28.5
MG1655	17.1 < MIC ≤ 34.1	0.89 < MIC ≤ 1.78 1.78 < MIC ≤ 3.56	MIC > 68.3	MIC > 28.5 MIC > 28.5	MIC > 68.3	MIC > 28.5 MIC > 28.5
S288C	MIC > 68.3	MIC > 28.5	MIC > 68.3	MIC > 28.5	MIC > 68.3	MIC > 28.5

xiii) MIC50, MIC90, MIC100 values for *Armillaria solidipes* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	$0.5 < MIC \leq 1$	$2.06 < MIC \leq 4.12$ $4.12 < MIC \leq 8.25$	$MIC > 8$	$MIC > 8.25$ $MIC > 8.25$	$MIC > 8$	$MIC > 8.25$ $MIC > 8.25$
<i>gyrA</i>	$1 < MIC \leq 2$	$2.06 < MIC \leq 4.12$ $2.06 < MIC \leq 4.12$	$MIC > 8$	$4.12 < MIC \leq 8.25$ $MIC > 8.25$	$MIC > 8$	$MIC > 8.25$ $MIC > 8.25$
MG1655	$1 < MIC \leq 2$	$1.03 < MIC \leq 2.06$ $2.06 < MIC \leq 4.12$	$MIC > 8$	$4.12 < MIC \leq 8.25$ $MIC > 8.25$	$MIC > 8$	$MIC > 8.25$ $MIC > 8.25$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	$1.46 < MIC \leq 2.93$	$1.21 < MIC \leq 2.42$ $1.21 < MIC \leq 2.42$	$11.75 < MIC \leq 23.5$	$19.4 < MIC \leq 38.8$ $MIC > 38.8$	$MIC > 23.5$	$MIC > 38.75$ $MIC > 38.75$
<i>gyrA</i>	$1.46 < MIC \leq 2.93$	$1.21 < MIC \leq 2.42$ $1.21 < MIC \leq 2.42$	$5.88 < MIC \leq 11.8$	$MIC > 38.8$ $MIC > 38.8$	$MIC > 23.5$	$MIC > 38.75$ $MIC > 38.75$
MG1655	$2.93 < MIC \leq 5.87$	$0.61 < MIC \leq 1.21$ $1.21 < MIC \leq 2.42$	$MIC > 23.5$	$19.4 < MIC \leq 38.8$ $MIC > 38.8$	$MIC > 23.5$	$MIC > 38.75$ $MIC > 38.75$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
5AC						
<i>marR</i>	$0.67 < MIC \leq 1.34$	$1.21 < MIC \leq 2.42$ $0.47 < MIC \leq 0.95$	$MIC > 21.5$	$19.4 < MIC \leq 38.8$ $15.3 < MIC \leq 30.5$	$MIC > 21.5$	$MIC > 38.75$ $MIC > 30.5$
<i>gyrA</i>	$0.67 < MIC \leq 1.34$	$1.21 < MIC \leq 2.42$ $0.95 < MIC \leq 1.9$	$MIC > 21.5$	$19.4 < MIC \leq 38.8$ $15.3 < MIC \leq 30.5$	$MIC > 21.5$	$MIC > 38.75$ $MIC > 30.5$
MG1655	$1.34 < MIC \leq 2.69$	$0.3 < MIC \leq 0.6$ $0.95 < MIC \leq 1.9$	$MIC > 21.5$	$19.4 < MIC \leq 38.8$ $15.3 < MIC \leq 30.5$	$MIC > 21.5$	$MIC > 38.75$ $MIC > 30.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	$1.17 < MIC \leq 2.34$	$0.92 < MIC \leq 1.84$ $0.92 < MIC \leq 1.84$	$9.37 < MIC \leq 18.8$	$14.8 < MIC \leq 29.5$ $MIC > 29.5$	$MIC > 18.75$	$MIC > 29.5$ $MIC > 29.5$
<i>gyrA</i>	$2.34 < MIC \leq 4.68$	$0.92 < MIC \leq 1.84$ $0.92 < MIC \leq 1.84$	$MIC > 18.8$	$14.8 < MIC \leq 29.5$ $MIC > 29.5$	$MIC > 18.75$	$MIC > 29.5$ $MIC > 29.5$
MG1655	$2.34 < MIC \leq 4.68$	$0.46 < MIC \leq 0.92$ $0.92 < MIC \leq 1.84$	$MIC > 18.8$	$14.8 < MIC \leq 29.5$ $MIC > 29.5$	$MIC > 18.75$	$MIC > 29.5$ $MIC > 29.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A

ix) MIC50, MIC90, MIC100 values for *Clitocybe nuda* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	$1.93 < MIC \leq 3.88$	$3.4 < MIC \leq 6.8$	$MIC > 15.5$	$MIC > 54.4$	$MIC > 15.5$	$MIC > 54.4$
<i>gyrA</i>	$1.93 < MIC \leq 3.88$	$13.6 < MIC \leq 27.3$	$MIC > 15.5$	$MIC > 54.4$	$MIC > 15.5$	$MIC > 54.4$
MG1655	$1.93 < MIC \leq 3.88$	$6.81 < MIC \leq 13.6$	$MIC > 15.5$	$MIC > 54.4$	$MIC > 15.5$	$MIC > 54.4$
S288C	$1.19 < MIC < 2.38$	$4.69 < MIC \leq 9.38$	$1.19 < MIC \leq 2.38$	$9.38 < MIC < 18.8$	$2.38 < MIC \leq 4.75$	$9.38 < MIC \leq 18.8$
EC						
<i>marR</i>	$0.8 < MIC \leq 1.6$	$0.67 < MIC \leq 1.34$	$MIC > 25.8$	$10.8 < MIC < 21.5$	$MIC > 25.8$	$10.8 < MIC \leq 21.5$
<i>gyrA</i>	$0.8 < MIC \leq 1.6$	$1.34 < MIC \leq 2.69$	$MIC > 25.8$	$10.8 < MIC < 21.5$	$MIC > 25.8$	$10.8 < MIC \leq 21.5$
MG1655	$0.8 < MIC \leq 1.6$	$0.67 < MIC \leq 1.34$	$MIC > 25.8$	$10.8 < MIC < 21.5$	$MIC > 25.8$	$10.8 < MIC \leq 21.5$
S288C	$3.22 < MIC \leq 6.44$	$4.19 < MIC < 8.38$	$6.44 < MIC < 12.9$	$4.19 < MIC \leq 8.38$	$6.44 < MIC \leq 12.9$	$8.38 < MIC \leq 16.8$
5AC						
<i>marR</i>	$0.51 < MIC \leq 1.03$	$0.88 < MIC \leq 1.75$	$8.25 < MIC \leq 16.5$	$14 < MIC < 28$	$MIC > 16.5$	$14 < MIC \leq 28$
<i>gyrA</i>	$1.03 < MIC \leq 2.06$	$0.88 < MIC \leq 1.75$	$8.25 < MIC \leq 16.5$	$14 < MIC < 28$	$MIC > 16.5$	$14 < MIC \leq 28$
MG1655	$1.03 < MIC \leq 2.06$	$0.88 < MIC \leq 1.75$	$8.25 < MIC \leq 16.5$	$14 < MIC < 28$	$MIC > 16.5$	$14 < MIC \leq 28$
S288C	$MIC > 16.5$	$7 < MIC \leq 14$	$MIC > 16.5$	$14 < MIC < 28$	$MIC > 16.5$	$14 < MIC \leq 28$
UI						
<i>marR</i>	$1.03 < MIC \leq 2.06$	$0.78 < MIC \leq 1.56$	$MIC > 16.5$	$MIC > 12.5$	$MIC > 16.5$	$MIC > 12.5$
<i>gyrA</i>	$2.06 < MIC \leq 4.13$	$1.56 < MIC \leq 3.13$	$MIC > 16.5$	$MIC > 12.5$	$MIC > 16.5$	$MIC > 12.5$
MG1655	$1.03 < MIC \leq 2.06$	$0.78 < MIC \leq 1.56$	$MIC > 16.5$	$MIC > 12.5$	$MIC > 16.5$	$MIC > 12.5$
S288C	$MIC > 16.5$	$MIC > 12.5$	$MIC > 16.5$	$MIC > 12.5$	$MIC > 16.5$	$MIC > 12.5$

x) MIC50, MIC90, MIC100 values for *Hypsizygus tessellatus* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC 50 (range) mycelia extract	MIC 50 (range) media extract	MIC 90 (range) mycelia extract	MIC 90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	ND $3.5 < MIC \leq 7$	$2 < MIC \leq 4$ $4 < MIC \leq 8$	ND $MIC > 14$	$MIC > 16$ $MIC > 16$	ND $MIC > 14$	$MIC > 16$ $MIC > 16$
<i>gyrA</i>	ND $3.5 < MIC \leq 7$	$2 < MIC \leq 4$ $4 < MIC \leq 8$	ND $MIC > 14$	$MIC > 16$ $MIC > 16$	ND $MIC > 14$	$MIC > 16$ $MIC > 16$
MG1655	ND $3.5 < MIC \leq 7$	$2 < MIC \leq 4$ $4 < MIC \leq 8$	ND $MIC > 14$	$MIC > 16$ $MIC > 16$	ND $MIC > 14$	$MIC > 16$ $MIC > 16$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	$2.38 < MIC \leq 4.75$ $2.36 < MIC \leq 4.7$	$9.69 < MIC \leq 19.4$ $1 < MIC \leq 2$	$MIC \leq 9.5$ $MIC > 9.4$	$MIC \leq 38.8$ $MIC > 16$	$MIC > 9.5$ $MIC > 9.4$	$MIC > 38.8$ $MIC > 16$
<i>gyrA</i>	$2.38 < MIC \leq 4.75$ $4.75 < MIC \leq 9.4$	$9.68 < MIC \leq 19.4$ $2 < MIC \leq 4$	$MIC > 9.5$ $MIC > 9.4$	$MIC > 38.8$ $MIC > 16$	$MIC > 9.5$ $MIC > 9.4$	$MIC > 38.8$ $MIC > 16$
MG1655	$4.75 < MIC \leq 9.5$ $4.75 < MIC \leq 9.4$	$19.4 < MIC \leq 38.8$ $1 < MIC \leq 2$	$MIC \leq 9.5$ $MIC > 9.4$	$MIC > 38.8$ $MIC > 16$	$MIC > 9.5$ $MIC > 9.4$	$MIC > 38.8$ $MIC > 16$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
5AC						
<i>marR</i>	$MIC \leq 29.5$ $7.4 < MIC \leq 14.8$	$1.77 < MIC \leq 3.5$ $1.8 < MIC \leq 3.5$	$MIC > 29.5$ $MIC > 29.5$	$MIC > 28.3$ $MIC > 28.3$	$MIC > 29.5$ $MIC > 29.5$	$MIC > 28.3$ $MIC > 28.3$
<i>gyrA</i>	$MIC > 29.5$ $7.4 < MIC \leq 14.8$	$1.77 < MIC \leq 3.5$ $1.8 < MIC \leq 3.5$	$MIC > 29.5$ $MIC > 29.5$	$MIC > 28.3$ $MIC > 28.3$	$MIC > 29.5$ $MIC > 29.5$	$MIC > 28.3$ $MIC > 28.3$
MG1655	ND $7.4 < MIC \leq 14.8$	$3.5 < MIC \leq 7$ $1.8 < MIC \leq 3.5$	$MIC > 29.5$ $MIC > 29.5$	$MIC > 28.3$ $MIC > 28.3$	$MIC > 29.5$ $MIC > 29.5$	$MIC > 28.3$ $MIC > 28.3$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	$7.5 < MIC \leq 15$ $7.5 < MIC \leq 15$	$MIC > 28.8$ $1.8 < MIC \leq 3.6$	$MIC > 15$ ND	$MIC > 28.8$ $MIC > 28.8$	$MIC > 15$ ND	$MIC > 28.8$ $MIC > 28.8$
<i>gyrA</i>	$7.5 < MIC \leq 15$ $7.5 < MIC \leq 15$	$MIC > 28.8$ $1.8 < MIC \leq 3.6$	$MIC > 15$ $MIC > 15$	$MIC > 28.8$ $MIC > 28.8$	$MIC > 15$ $MIC > 15$	$MIC > 28.8$ $MIC > 28.8$
MG1655	$7.5 < MIC \leq 15$ $MIC > 15$	$MIC > 28.8$ $1.8 < MIC \leq 3.6$	$MIC > 15$ $MIC > 15$	$MIC > 28.8$ $MIC > 28.8$	$MIC > 15$ $MIC > 15$	$MIC > 28.8$ $MIC > 28.8$
S288C	N/A	N/A	N/A	N/A	N/A	N/A

xi) MIC50, MIC90, MIC100 values for *Ganoderma tsugae* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S. cerevisiae* S288C. Ranges were recorded in format $X < \text{MIC} \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < \text{MIC} < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC50 (range) mycelia extract	MIC50 (range) media extract	MIC90 (range) mycelia extract	MIC90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	$2.94 < \text{MIC} \leq 5.88$	$5 < \text{MIC} \leq 10$	$\text{MIC} > 11.8$	$\text{MIC} > 40$	$\text{MIC} > 11.8$	$\text{MIC} > 40$
<i>gyrA</i>	$1.47 < \text{MIC} \leq 2.94$	$2.5 < \text{MIC} \leq 5$	$\text{MIC} > 11.8$	$\text{MIC} > 40$	$\text{MIC} > 11.8$	$\text{MIC} > 40$
MG1655	$1.47 < \text{MIC} \leq 2.94$	$2.5 < \text{MIC} \leq 5$	$\text{MIC} > 11.8$	$\text{MIC} > 40$	$\text{MIC} > 11.8$	$\text{MIC} > 40$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	ND	$1.02 < \text{MIC} \leq 2.03$	$3.5 < \text{MIC} \leq 7$	$\text{MIC} > 16.3$	$\text{MIC} > 7$	$\text{MIC} > 16.3$
<i>gyrA</i>	$0.44 < \text{MIC} \leq 0.88$	$1.02 < \text{MIC} \leq 2.03$	$3.5 < \text{MIC} \leq 7$	$\text{MIC} > 16.3$	$\text{MIC} > 7$	$\text{MIC} > 16.3$
MG1655	$0.44 < \text{MIC} \leq 0.88$	$0.51 < \text{MIC} \leq 1.02$	$3.5 < \text{MIC} \leq 7$	$\text{MIC} > 16.3$	$\text{MIC} > 7$	$\text{MIC} > 16.3$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
5AC						
<i>marR</i>	$0.875 < \text{MIC} \leq 1.75$	$0.77 < \text{MIC} \leq 1.55$	$\text{MIC} > 14$	$\text{MIC} > 24.8$	$\text{MIC} > 14$	$\text{MIC} > 24.8$
<i>gyrA</i>	$0.875 < \text{MIC} \leq 1.75$	$1.55 < \text{MIC} \leq 3.09$	$\text{MIC} > 14$	$\text{MIC} > 24.8$	$\text{MIC} > 14$	$\text{MIC} > 24.8$
MG1655	$0.875 < \text{MIC} \leq 1.75$	$0.77 < \text{MIC} \leq 1.55$	$\text{MIC} > 14$	$\text{MIC} > 24.8$	$\text{MIC} > 14$	$\text{MIC} > 24.8$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	$1.56 < \text{MIC} \leq 3.13$	$2.75 < \text{MIC} \leq 5.5$	$\text{MIC} > 12.5$	$11 < \text{MIC} \leq 22$	$\text{MIC} > 12.5$	$\text{MIC} > 22$
<i>gyrA</i>	$1.56 < \text{MIC} \leq 3.13$	$1.38 < \text{MIC} \leq 2.75$	$\text{MIC} > 12.5$	$11 < \text{MIC} \leq 22$	$\text{MIC} > 12.5$	$\text{MIC} > 22$
MG1655	$1.56 < \text{MIC} \leq 3.13$	$2.75 < \text{MIC} \leq 5.5$	$\text{MIC} > 12.5$	$\text{MIC} > 22$	$\text{MIC} > 12.5$	$\text{MIC} > 22$
S288C	N/A	N/A	N/A	N/A	N/A	N/A

xii) MIC50, MIC90, MIC100 values for *Ganoderma lucidum* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC50 (range) mycelia extract	MIC50 (range) media extract	MIC90 (range) mycelia extract	MIC90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	$3.5 < MIC \leq 7$	$4.38 < MIC \leq 8.75$	$MIC > 14$	$MIC > 8.75$	$MIC > 14$	$MIC > 8.75$
<i>gyrA</i>	$7 < MIC \leq 14$	$1.09 < MIC \leq 2.18$	$MIC > 14$	$MIC > 8.75$	$MIC > 14$	$MIC > 8.75$
MG1655	$3.5 < MIC \leq 7$	$2.18 < MIC \leq 4.38$	$MIC > 14$	$MIC > 8.75$	$MIC > 14$	$MIC > 8.75$
S288C	$MIC > 14$	$MIC > 8.75$	$MIC > 14$	$MIC > 8.75$	$MIC > 14$	$MIC > 8.75$
EC						
<i>marR</i>	$1.75 < MIC \leq 3.5$	$0.35 < MIC \leq 0.7$	$7 < MIC \leq 14$	$11.1 < MIC \leq 22.3$	$MIC > 14$	$MIC > 22.3$
<i>gyrA</i>	$3.5 < MIC \leq 7$	$0.7 < MIC \leq 1.4$	$7 < MIC \leq 14$	$11.1 < MIC \leq 22.3$	$MIC > 14$	$MIC > 22.3$
MG1655	$3.5 < MIC \leq 7$	$0.7 < MIC \leq 1.4$	$7 < MIC \leq 14$	$11.1 < MIC \leq 22.3$	$MIC > 14$	$MIC > 22.3$
S288C	$MIC > 14$	$MIC > 22.3$	$MIC > 14$	$MIC > 22.3$	$MIC > 14$	$MIC > 22.3$
5AC						
<i>marR</i>	$2.34 < MIC \leq 4.69$	$0.37 < MIC \leq 0.73$	$9.38 < MIC \leq 18.8$	$5.88 < MIC \leq 11.8$	$MIC > 18.8$	$MIC > 11.8$
<i>gyrA</i>	$2.34 < MIC \leq 4.69$	$0.73 < MIC \leq 1.47$	$9.38 < MIC \leq 18.8$	$5.88 < MIC \leq 11.8$	$MC > 18.8$	$MIC > 11.8$
MG1655	$2.34 < MIC \leq 4.69$	$0.37 < MIC \leq 0.73$	$4.69 < MIC \leq 9.38$	$MIC > 11.8$	$MIC > 18.8$	$MIC > 11.8$
S288C	$MIC > 18.8$	$MIC > 12$	$MIC > 18.8$	$MIC > 12$	$MIC > 18.8$	$MIC > 12$
UI						
<i>marR</i>	$1.47 < MIC \leq 2.94$	$1.11 < MIC \leq 2.22$	$5.88 < MIC \leq 11.8$	$17.8 < MIC \leq 35.5$	$MIC > 11.8$	$MIC > 35.5$
<i>gyrA</i>	$1.47 < MIC \leq 2.94$	$1.11 < MIC \leq 2.22$	$5.88 < MIC \leq 11.8$	$17.8 < MIC \leq 35.5$	$MIC > 11.8$	$MIC > 35.5$
MG1655	$1.47 < MIC \leq 2.94$	$1.11 < MIC \leq 2.22$	$2.94 < MIC \leq 5.88$	$17.8 < MIC \leq 35.5$	$MIC > 11.8$	$MIC > 35.5$
S288C	$MIC > 11.8$	$MIC > 35.5$	$MIC > 11.8$	$MIC > 35.5$	$MIC > 11.8$	$MIC > 35.5$

xiii) MIC50, MIC90, MIC100 values for *Grifola frondosa* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC50 (range) mycelia extract	MIC50 (range) media extract	MIC90 (range) mycelia extract	MIC90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	ND $7.13 < MIC \leq 14.25$	ND $MIC > 30.5$	ND $MIC > 14.25$	ND $MIC > 30.5$	ND $MIC > 14.25$	ND $MIC > 30.5$
<i>gyrA</i>	ND $3.56 < MIC \leq 7.13$	ND $15.25 < MIC \leq 30.5$	ND $MIC > 14.25$	ND $MIC > 30.5$	ND $MIC > 14.25$	ND $MIC > 30.5$
MG1655	ND $7.13 < MIC \leq 14.25$	ND $15.25 < MIC \leq 30.5$	ND $MIC > 14.25$	ND $MIC > 30.5$	ND $MIC > 14.25$	ND $MIC > 30.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	$1.19 < MIC \leq 2.38$ $5.5 < MIC \leq 11$	$13.5 < MIC \leq 27$ $0.95 < MIC \leq 1.9$	$MIC > 9.5$ $MIC > 22$	$MIC > 54$ $15.25 < MIC \leq 30.5$	$MIC > 9.5$ $MIC > 22$	$MIC > 54$ $MIC > 30.5$
<i>gyrA</i>	$2.38 < MIC \leq 2.75$ $5.5 < MIC \leq 11$	$13.5 < MIC \leq 27$ $7.6 < MIC \leq 15.25$	$MIC > 9.5$ $MIC > 22$	$MIC > 54$ $15.25 < MIC \leq 30.5$	$MIC > 9.5$ $MIC > 22$	$MIC > 54$ $MIC > 30.5$
MG1655	ND $2.75 < MIC \leq 5.5$	$13.5 < MIC \leq 27$ $0.95 < MIC \leq 1.9$	ND $MIC > 22$	$27 < MIC \leq 54$ $15.25 < MIC \leq 30.5$	ND $MIC > 22$	$MIC > 54$ $MIC > 30.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
5AC						
<i>marR</i>	$1.19 < MIC \leq 2.38$ $0.59 < MIC \leq 1.19$	$1.17 < MIC \leq 2.34$ $1.02 < MIC \leq 2.04$	$MIC > 4.75$ $2.38 < MIC \leq 4.75$	$18.75 < MIC \leq 37.5$ $MIC > 32.75$	$MIC > 4.75$ $MIC > 4.75$	$MIC > 37.5$ $MIC > 32.75$
<i>gyrA</i>	$2.38 < MIC \leq 4.75$ $0.29 < MIC \leq 0.59$	$1.17 < MIC \leq 2.34$ $1.02 < MIC \leq 2.04$	$MIC > 4.75$ $2.38 < MIC \leq 4.75$	$18.75 < MIC \leq 37.5$ $MIC > 32.75$	$MIC > 4.75$ $MIC > 4.75$	$MIC > 37.5$ $MIC > 32.75$
MG1655	ND $0.29 < MIC \leq 0.59$	$2.34 < MIC \leq 4.68$ $1.02 < MIC \leq 2.04$	ND $2.38 < MIC \leq 4.75$	ND $MIC > 32.75$	ND $MIC > 4.75$	ND $MIC > 32.75$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	$MIC > 4.75$ $1.32 < MIC \leq 2.65$	$1.37 < MIC \leq 2.73$ $1.17 < MIC \leq 2.34$	$MIC > 4.75$ $10.6 < MIC \leq 21.25$	$21.88 < MIC \leq 43.75$ $MIC > 37.5$	$MIC > 4.75$ $MIC > 21.25$	$MIC > 43.75$ $MIC > 37.5$
<i>gyrA</i>	$MIC > 4.75$ $2.65 < MIC \leq 5.3$	$1.37 < MIC \leq 2.73$ $1.17 < MIC \leq 2.34$	$MIC > 4.75$ $10.6 < MIC \leq 21.25$	$21.88 < MIC \leq 43.75$ $MIC > 37.5$	$MIC > 4.75$ $MIC > 21.25$	$MIC > 43.75$ $MIC > 37.5$
MG1655	ND $2.65 < MIC \leq 5.3$	$1.37 < MIC \leq 2.73$ $1.17 < MIC \leq 2.34$	ND $10.6 < MIC \leq 21.25$	ND $MIC > 37.5$	ND $MIC > 21.25$	ND $MIC > 37.5$
S288C	N/A	N/A	N/A	N/A	N/A	N/A

xiv) MIC50, MIC90, MIC100 values for *Boletus edulis* extracts against microbes *E. coli marR*, *gyrA* and wild-type MG1655, and *S cerevisiae* S288C. Ranges were recorded in format $X < MIC \leq Y$ where Y is the lowest concentration (mg/ml) where 50%, 90% or 100% growth inhibition is observed and X is the next lower concentration tested. In some cases, the ranges are formatted $X < MIC < Y$ where the MIC lies within the range but not equal to either of the known concentrations. ND= MIC was not determined. Two entries are provided in cases where two independent experiments were done. LN=low nitrogen induced sample, EC= spent *E. coli* induced sample, 5AC= 5-azacytidine induced treatment, and UI= un-induced.

	MIC50 (range) mycelia extract	MIC50 (range) media extract	MIC90 (range) mycelia extract	MIC90 (range) media extract	MIC100 (range) mycelia extract	MIC100 (range) media extract
LN						
<i>marR</i>	$0.6 < MIC \leq 1.2$	$1.17 < MIC \leq 2.34$	$MIC > 4.75$	$MIC > 18.8$	$MIC > 4.75$	$MIC > 18.8$
<i>gyrA</i>	$0.6 < MIC \leq 1.2$	$1.18 < MIC \leq 2.34$	$MIC > 4.75$	$MIC > 18.8$	$MIC > 4.75$	$MIC > 18.8$
MG1655	$0.6 < MIC \leq 1.2$	$2.34 < MIC \leq 4.69$	$MIC > 4.75$	$MIC > 18.8$	$MIC > 4.75$	$MIC > 18.8$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
EC						
<i>marR</i>	$0.3 < MIC \leq 0.6$	$1.6 < MIC \leq 3.2$	$MIC > 4.75$	$MIC > 25.8$	$MIC > 4.75$	$MIC > 25.8$
<i>gyrA</i>	$1.19 < MIC \leq 2.38$	$1.6 < MIC \leq 3.2$	$MIC > 4.75$	$MIC > 25.8$	$MIC > 4.75$	$MIC > 25.8$
MG1655	$1.19 < MIC \leq 2.38$	$0.8 < MIC \leq 1.6$	$MIC > 4.75$	$MIC > 25.8$	$MIC > 4.75$	$MIC > 25.8$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
5AC						
<i>marR</i>	$0.59 < MIC \leq 1.19$	$1.61 < MIC \leq 3.22$	$MIC > 4.75$	$12.8 < MIC \leq 25.8$	$MIC > 4.75$	$MIC > 25.8$
<i>gyrA</i>	$1.19 < MIC \leq 2.38$	$1.61 < MIC \leq 3.22$	$MIC > 4.75$	$12.8 < MIC \leq 25.8$	$MIC > 4.75$	$MIC > 25.8$
MG1655	$0.59 < MIC \leq 1.19$	$0.8 < MIC \leq 1.6$	$MIC > 4.75$	$12.8 < MIC \leq 25.8$	$MIC > 4.75$	$MIC > 25.8$
S288C	N/A	N/A	N/A	N/A	N/A	N/A
UI						
<i>marR</i>	$0.3 < MIC \leq 0.6$	$1.78 < MIC \leq 3.53$	$MIC > 4.75$	$MIC > 28.3$	$MIC > 4.75$	$MIC > 28.3$
<i>gyrA</i>	$1.19 < MIC \leq 2.38$	$1.78 < MIC \leq 3.53$	$MIC > 4.75$	$MIC > 28.3$	$MIC > 4.75$	$MIC > 28.3$
MG1655	$0.3 < MIC \leq 0.6$	$0.88 < MIC \leq 1.78$	$MIC > 4.75$	$MIC > 28.3$	$MIC > 4.75$	$MIC > 28.3$
S288C	N/A	N/A	N/A	N/A	N/A	N/A

Appendix III-Induced vs Un-Induced Figures

a)

b)

Figure i. Comparison between the susceptibility of the three bacterial strains *marR* R77H, *gyrA* S83L and wild-type MG1655 to the extracts of the 'un-induced' fungal cultures. Data is shown for all fourteen fungal species, seven of each shown in figures (a) and (b). Inverse MIC of the concentrations at MIC₅₀, MIC₉₀, MIC₁₀₀ inhibition [1/MIC (mg/ml)] are plotted for both the mycelial extracts and the media extracts.

Figure ii. Comparison between the susceptibility of the three bacterial strains *marR* R77H, *gyrA* S83L and wild-type MG1655 to extracts of cultures treated with the low-nutrient medium induction. Data is shown for all fourteen fungal species, seven of each shown in figures (a) and (b). Inverse MIC of the concentrations at MIC₅₀, MIC₉₀, MIC₁₀₀ [1/MIC (mg/ml)] are plotted for both the mycelial extracts and the media extracts.

Figure iii. Comparison between the susceptibility of the three bacterial strains *marR* R77H, *gyrA* S83L and wild-type MG1655 to extracts of cultures treated with spent *E. coli* induction. Data is shown for all fourteen fungal species, seven of each shown in figures (a) and (b). Inverse MIC of the concentrations at MIC₅₀, MIC₉₀, MIC₁₀₀ [1/MIC (mg/ml)] are plotted for both the mycelial extracts and the media extracts.

a)

b)

Figure iv. Comparison between the susceptibility of the three bacterial strains *marR* R77H, *gyrA* S83L and wild-type MG1655 to extract of cultures treated with 5-azacytidine induction. Data is shown for all fourteen fungal species, seven of each shown in figures (a) and (b). Inverse MIC of the concentrations at MIC₅₀, MIC₉₀, MIC₁₀₀ [1/MIC (mg/ml)] are plotted for both the mycelial extracts and the media extracts.