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# **Growth and Therapeutic Properties of *Agaricus blazei***



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**Doctor of Philosophy**

By

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This is dedicated to Mum, Oma, Nana, and Opa

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## Abstract

*Agaricus blazei* is an immensely popular edible “medicinal mushroom” in Japan, mainly due to traditional beliefs that it has antitumour properties, and the ability to stimulate the immune system. The majority of scientific research carried out on *A. blazei* has demonstrated that polysaccharides extracted from mushrooms fruiting bodies are the active agents for the purported anticancer properties. Limited research has been undertaken with regards to liquid-cultured mycelium and liquid culture filtrates of *A. blazei* and this investigation involved screening these products for novel medicinal properties.

The growth of the fungus was examined on solid agar for the propagation of mycelium which was used for inoculation of liquid cultures. Optimal fungal storage conditions were also determined. The optimum growth temperature and pH of *A. blazei* on yeast malt agar were 28-29 °C and pH 5-6, respectively. Storage of mycelial plugs at -80°C in 10% glycerol was found to maintain viability of the fungus for up to 36 months. Subsequent growth trials in liquid media found that a temperature of 30°C over a pH range of 4-8 were optimal for mycelial growth. Glucose as a carbohydrate source produced the most mycelium, while sucrose was most favourable for exopolysaccharide production. The exopolysaccharides produced were identified as mannan-protein complexes.

Organic solvent extracts of liquid-cultured mycelia were examined, and antibacterial activity was identified against *Branhamella catarhalis* and some Gram-positive bacteria, particularly for dichloromethane and ethyl acetate extracts. *In vitro* cytotoxic activity was observed against cervical cancer and lymphoma cell lines, particularly for hexane and dichloromethane extracts. Protein extracts of *Agaricus blazei* liquid-cultured mycelium were found to have antiviral effect against simian rotavirus, although the cause of the observed effect was likely due to the presence of a trypsin inhibitor in the extract. Cytotoxic activity of the protein extract was also identified against lymphoma cells *in vitro*. Exopolysaccharides from liquid culture filtrate of *A. blazei* did not have an effect on rotavirus, but had a marked cytotoxic effect on lymphoma cells, and to a lesser extent of cervical cancer cells. Thus, the cytotoxic effect was found to be specific for tumour cells.

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## Declaration

I hereby declare, that to the best of my knowledge, this thesis contains neither material which has been accepted for the award to the candidate of any other degree or diploma, or any material previously published or written by another person, except where due reference is made in the text of the thesis. Where the work is based on joint research or publications, the thesis discloses the relative contributions of the respective workers or authors.

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Danielle R. Tilmanis

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## List of Abbreviations

Standard chemical symbols and SI units are used without definition.

<b>°C</b>	Degrees Celsius
<b>ω-6APFA</b>	Omega -6 polyunsaturated fatty acid
<b>%V</b>	Percentage cell viability
<b>1xEPS</b>	Exopolysaccharide extract precipitated with an equal volume of ethanol
<b>2xEPS</b>	Exopolysaccharide extract precipitated with twice the volume of ethanol
<b>5-FU</b>	5-fluorouracil
<b>ABM</b>	<i>Agaricus blazei</i> Murrill
<b>ABWE</b>	Water extract of <i>Agaricus blazei</i>
<b>ACM</b>	Australian Collection of Micro-organisms
<b>ADH</b>	Alcohol dehydrogenase
<b>AGS</b>	Human stomach cancer cell line
<b>ASC</b>	<i>Agaricus blazei</i> spent compost
<b>ATCC</b>	American Type Culture Collection
<b>BHI</b>	Brain heart infusion broth
<b>BM</b>	Basal medium
<b>BRM</b>	Biological response modifier
<b>BSA</b>	Bovine serum albumin
<b>CC<sub>50</sub></b>	Cytotoxic concentration 50
<b>CDA</b>	Czapek dox agar
<b>CDA</b>	Corn meal agar
<b>CMM</b>	Complete mushroom media
<b>CO<sub>2</sub></b>	Carbon dioxide
<b>CPE</b>	Cytopathic effect
<b>DCM</b>	Dichloromethane
<b>DEAE</b>	Diethylaminoethyl
<b>dH<sub>2</sub>O</b>	Distilled water
<b>DMEM</b>	Dulbecco's modified Eagle's medium
<b>DMSO</b>	Dimethylsulphoxide
<b>DNA</b>	Deoxyribonucleic acid
<b>DPPH</b>	1, 1-diphenyl-2-picrylhydrazyl
<b>EC<sub>50</sub></b>	Effective concentration 50
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>EtOAc</b>	Ethyl acetate
<b>EPS</b>	Exo-polysaccharide
<b>FBS</b>	Foetal bovine serum

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<b>FMDV</b>	Foot and mouth disease virus
<b>F-PDA</b>	Fresh potato dextrose agar
<b>GABA</b>	Gamma-aminobutyric acid
<b>GLUC</b>	Glucose liquid media
<b>HBA</b>	Horse blood agar
<b>HBV</b>	Hepatitis B virus
<b>HCl</b>	Hydrochloric acid
<b>HCV</b>	Hepatitis C virus
<b>HIV</b>	Human immunodeficiency virus
<b>HSV</b>	Herpes simplex virus
<b>IC<sub>50</sub></b>	Inhibitory concentration 50
<b>IL-1</b>	Interleukin 1
<b>IL-6</b>	Interleukin 6
<b>IL-8</b>	Interleukin 8
<b>MEA</b>	Malt extract agar
<b>MEB</b>	Malt extract broth
<b>MEM</b>	Minimum Essential medium
<b>MeOH</b>	Methanol
<b>MeOH-P</b>	Methanol extract precipitate
<b>MeOH-S</b>	Methanol extract supernatant
<b>MHA</b>	Mueller-Hinton agar
<b>MHB</b>	Mueller-Hinton broth
<b>MIC</b>	Minimal Inhibitory Concentration
<b>MS</b>	Mass spectrometry
<b>MTT</b>	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
<b>MW</b>	Molecular weight
<b>NA</b>	Nutrient agar
<b>NB</b>	Nutrient broth
<b>NaCl</b>	Sodium Chloride
<b>NaOH</b>	Sodium hydroxide
<b>NMR</b>	Nuclear magnetic resonance spectroscopy
<b>OD</b>	Optical density
<b>PAGE</b>	Polyacrlamide gel electrophoresis
<b>PBS</b>	phosphate buffered saline
<b>PDA</b>	Potato dextrose agar
<b>PDB</b>	Potato dextrose broth
<b>PMP</b>	PDB-malt-peptone broth
<b>p-s</b>	penicillin-streptomycin solution
<b>PSK</b>	Krestin
<b>PSPC</b>	Polysaccharide-protein complex

<b>RNA</b>	Ribonucleic acid
<b>rpm</b>	Revolutions per minute
<b>RPMC</b>	Rat peritoneal mast cells
<b>RPMI</b>	RPMI 1640 cell media formulation
<b>SDA</b>	Sabouraud dextrose agar
<b>SDS</b>	Sodium dodecyl sulphate
<b>SF-MEM</b>	serum-free minimum essential media
<b>SF-MEM-T</b>	serum-free minimum essential media + porcine trypsin
<b>SMA</b>	Sabouraud maltose agar
<b>SUCR</b>	Sucrose liquid media
<b>TLC</b>	Thin layer chromatography
<b>TNF-<math>\alpha</math></b>	Tumour necrosis factor alpha
<b>WA</b>	Wort agar
<b>WEE</b>	Western equine encephalitis
<b>YMA</b>	Yeast malt agar
<b>YMB</b>	Yeast malt broth