THE SUBMERGED-CULTURE FERMENTATION OF *CORIOLUS VERSICOLOR* IN MILK PERMEATE BASED MEDIA AND THE CHARACTERISATION OF ITS BIOACTIVE POLYSACCHARIDES

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ABSTRACT

The protein-bound polysaccharides of *Coriolus versicolor* (CPS) are known to improve human immune functions against cancers and various infectious diseases. With the increasing concern for longer lifespan and better quality of life, CPS may find new application in the food industry as a novel nutriceutical. The commercial therapeutic CPS are the intracellular polysaccharides of *Coriolus versicolor* (IPS) produced either through the submerged-culture fermentation or from the extract of the fruit body of *Coriolus versicolor*. The objective of this study was to characterise the IPS and EPS (the extracellular polysaccharides) produced by *C. versicolor* through submerged-culture fermentation that was based on cost-effective milk permeate (MP) as a medium component. Ten *C. versicolor* species were screened and the strain Wr-74 was found to be the most comparable to the patented ATCC-20545 strain in terms of EPS/IPS production, morphology of the fruit bodies and weight-average molecular weight distributions of EPS/IPS. In addition, *in vitro* physiological activities of EPS, IPS and mushroom extracts on cytokine production were investigated using murine splenocytes.

The growth medium was optimised using MP as a base component, indicating the 50%MP-YEG medium was comparable to YEG medium in terms of EPS/IPS levels. When the lactose in MP was hydrolysed (HMP) by 0.04 % (v/v) Maxilact® lactase, a 40-60 % increase in EPS/IPS levels was observed in the 50%HMP-YEG medium compared to 50%MP-YEG medium. Approximately twice the amounts of EPS (3.2 mg/mL) and IPS (0.3 mg/mL) were obtained in the 100%HMP-YEG medium compared to the 50%HMP-YEG medium. Different nitrogen sources were screened and yeast extract supplied by DIFCO was found to be the most suitable for EPS/IPS production. Though glucose was the main carbon source consumed, excess amount of glucose would retard EPS/IPS production. The optimum carbon to nitrogen ratio (C/N) ratio was approximately 70.

Submerged-culture fermentation was conducted using a modified *impeller-assistant airlift fermenter*. This fermenter was equipped with an inner draught tube and helical impeller to produce an efficient circulation of broth and dissolved oxygen.

Wr-74 produced higher levels of EPS than ATCC-20545 in the fermenter. An approximately 74 % increase in EPS level was obtained in the 50%HMP-YEG medium compared to 50%MP-YEG medium. The EPS levels were close to those obtained in the shake flasks (~2.5 mg/mL). The effects of antifoaming agents and salt addition were also investigated in the fermenter. Agitation with the helical impeller appeared to improve the EPS production, but resulted in lower biomass levels.

Studies on the EPS/IPS compositions using HPLC indicated that the EPS from both strains only contained glucose. However, the IPS probably contained galactose, mannose or/and xylose in addition to glucose. Ratios of polysaccharide to protein were approximately 15:1 and 25:1 for EPS from ATCC-20545 and Wr-74, respectively. The EPS from both strains contained about 80 % of pure polysaccharides. Approximately 82 % of IPS and 32 % of EPS from both strains could be obtained in the water-soluble form. Results of the amino acid analysis showed that the IPS from both strains contained higher levels of amino acids (16 %, w/w) than the EPS (2 % to 4 %, w/w). The EPS and IPS from both strains had very similar molecular weight distributions. The weight-average molecular weights (Mw) of IPS from both strains were approximately ten times higher (10^5 Da) than EPS (10^4 Da) in the major elution range of 9.8 to 10.3 mL. The largest Mw fractions of EPS and IPS from both strains were about 10^6 Da in the elution range of 8.2 to 8.7 mL.

All samples of EPS, IPS and mushroom extracts stimulated cytokine production from murine splenocytes. Generally, lower polysaccharide levels (0.1 to 2.0 μg/mL) induced higher levels of cytokines. The mushroom extract from ATCC-20545 induced higher levels of IL-12 and IFN-γ than that from Wr-74. The IPS from the mycelia of Wr-74 induced higher IL-12 production, but lower levels of IFN-γ than that from ATCC-20545. The EPS from both strains were comparable in terms of IL-12 and IFN-γ production.

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CHAPTER I INTRODUCTION

The biopolymers produced by *Coriolus versicolor* are protein-bound polysaccharides (CPS), known to function as anticancer agents, immunopotentiators and biological response modifiers (Cho et al., 1988; Ueno et al., 1989; Sakagami et al., 1991; Wang et al., 1996; Yang et al., 1992a; Fisher and Yang, 2002). Belonging to the higher class of fungi-*Basidiomycetes*, *Coriolus versicolor* is a medicinal mushroom known for over a thousand years in the East. Its fruit bodies grow on tree trunks and stumps all year round in the temperate zones throughout the world. Of more than 200 medicinal mushrooms, the polysaccharides from *C. versicolor* are commercially the most established (Johl et al., 1996). In China and Japan, *C. versicolor* mushrooms were dried and the extracts were obtained either by decoction or by grinding the fruit-body into powder to be used as a herbal tea. During the 1970s and the 1980s, Japanese and Chinese scientists discovered the biological activities of *C. versicolor* extracts and began an extensive controlled clinical research on *C. versicolor* polysaccharides.

PSK (polysaccharides-Krestin) were the first commercial CPS preparation of *C. versicolor*, produced by Kureha Chemical Industries Co. Ltd in Japan through batch submerged-culture fermentation. Subsequently, another similar product called PSP was isolated by Chinese scientists. Both PSK and PSP were extracted from *C. versicolor* mycelia. PSK were produced from CM-101 and CM-103 (ATCC-20545, Hotta et al., 1981) and PSP was produced from Cov-1 strains of *C. versicolor* (Yang et al., 1992). All the strains used for the commercial production of CPS were patented.

Though the products have been marketed for years, their potential physiological activities and applications in the clinical area continued to interest scientists worldwide. In New Zealand, other medicinal mushroom polysaccharides are currently available on the market. However, they do not include *C. versicolor* polysaccharides. As a very promising health-care product, CPS would have broad applications in the food industry as a nutriceutical.

The objectives of the project are as follows:

- To select a local strain of *C. versicolor* that is comparable to the patented strain, ATCC-20545 through a screening process;
- To use milk permeate, a waste stream of the dairy industry, as a low cost base medium for CPS production;
- To optimize CPS production through the submerged-culture fermentation using a suitable bioreactor;
- To characterize the CPS isolated from the broth and biomass in terms of molecular weights and chemical compositions;
- To investigate the physiological activities of CPS based on cytokine production and the effect on cancer cell proliferation.