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Biotechnology Advances 21 (2003) 109–122
BIOTECHNOLOGY ADVANCES
www.elsevier.com/locate/biotechadv

Polysaccharopeptides of *Coriolus versicolor*: physiological activity, uses, and production

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Received 5 June 2002; accepted 8 January 2003

Abstract

The protein-bound polysaccharides or polysaccharopeptides produced by *Coriolus versicolor* are effective immunopotentiators, which are used to supplement the chemotherapy and radiotherapy of cancers and various infectious diseases. Antitumor activity of polysaccharopeptides has been documented. Several kinds of protein-bound polysaccharides have been shown to be produced by the white rot fungus, *C. versicolor*. Although some of these polymers are structurally distinct, they are not distinguishable in terms of their physiological activity. This review focuses on the physiologically active polysaccharopeptides of *C. versicolor*. In nature, *C. versicolor* occurs as a mushroom body, but the fungus can be grown as mycelial biomass in submerged culture in bioreactors. Mushrooms gathered in the wild, cultivated mushrooms, and the mycelial biomass of submerged culture are used to produce the polysaccharopeptides. Submerged cultures are typically carried out in batches lasting 5–7 days and at 25–27 °C. Hot water extraction of the biomass is used to recover the thermostable polysaccharopeptides that are concentrated, purified, and dried into a powder for medicinal use. In view of the documented physiological benefits of these compounds, extensive research is underway on the structure, composition, production methods, and use of new *C. versicolor* strains for producing the therapeutic biopolymers. Properties, physiological activity, recovery, and purification of the bioactive polysaccharopeptides are discussed.

Keywords: *Coriolus versicolor*; Antitumor polysaccharides; Polysaccharopeptides; Protein-bound polysaccharides

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0734-9750/03/$ - see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0734-9750(03)00002-8

1. Introduction

Medicinal mushrooms have an established history of use in traditional oriental therapies. Modern clinical practice in Japan, China, Korea, and other Asian countries continues to rely on mushroom-derived preparations. Medicinal effects have been demonstrated for many traditionally used mushrooms (Ooi and Liu, 1999), including extracts of *Favolus alveolarius* (Chang et al., 1988), *Phellinus linteus* (Chung et al., 1993; Kim et al., 2001), *Agaricus campestris* (Gray and Flatt, 1998), *Pestalotiopsis* sp. (Kiho et al., 1997), *Lentinus edodes* (Kim and Park, 1979; Sugano et al., 1985; Song et al., 1998), *Pleurotus ostreatus* (Kim and Park, 1979), *Tricholoma* sp. (Wang et al., 1995, 1996a; Liu et al., 1996), and *Coriolus versicolor* (Kim and Park, 1979; Mayer and Drews, 1980; Fujita et al., 1988; Li et al., 1990; Yang et al., 1992a; Han et al., 1996; Mao and Gridley, 1998; Ng, 1998; Ooi and Liu, 1999; Chu et al., 2002). Of the mushroom-derived therapeutics, polysaccharopeptides obtained from *C. versicolor* are commercially the best established. In addition to its medical applications, *C. versicolor* is widely used to degrade recalcitrant organic pollutants such as pentachlorophenol (PCP). Here we examine the properties, physiological activity, recovery, and purification of the bioactive polysaccharopeptides of *C. versicolor*.

Both extracellular and intracellular polysaccharopeptides of *C. versicolor* are physiologically active as biological response modifiers. In traditional medical practices of China and Japan, *C. versicolor* mushroom was harvested, dried, ground, and made into tea. Healing properties of *C. versicolor* extracts were noticed by Chinese and Japanese scientists and thus began an extensive controlled clinical research on *C. versicolor* extracts. Interestingly, the dose of the active polymers in the traditional tea was similar to that used in modern clinical practice. In nature, *C. versicolor* grows as a bracket or shelf mushroom; however, the fungus can be grown in submerged fermentation as mycelial biomass.

The best known commercial polysaccharopeptide preparations of *C. versicolor* are polysaccharopeptide Krestin (PSK) and polysaccharopeptide PSP. Both products are obtained from the extraction of *C. versicolor* mycelia. PSK and PSP are Japanese and Chinese products, respectively. Both products have similar physiological activities but are structurally different. PSK and PSP are produced from CM-101 and Cov-1 strains of *C. versicolor*, respectively. Both products are obtained by batch fermentation. PSK fermentation lasts up to 10 days, whereas PSP production involves a 64-h culture. PSK is recovered from hot water extracts of the biomass by salting out with ammonium sulfate, whereas PSP is recovered by alcoholic precipitation from the hot water extract.

PSK was commercialized by Kureha Chemicals, Japan. After extensive clinical trials, PSK was approved for use in Japan in 1977, and by 1985, it ranked 19th on the list of the world's most commercially successful drugs (Yang et al., 1992a). Annual Japanese sales of PSK in 1987 were worth US$357 million (Yang et al., 1992a). PSP appeared on the market about 10 years after PSK. In addition to clinically tested PSK and PSP, numerous other extract preparations of *C. versicolor* are on the market as neutraceuticals and traditional medicines. Neutraceutical polysaccharopeptide preparations are sold worldwide in the form of capsules, ground biomass tablets, syrups, food additives, and teas. Traditional usage, pharmacological activities, and clinical effects of *C. versicolor* preparations have been discussed by Chu et al. (2002).

2. *C. versicolor* mushroom

The visible form of *C. versicolor* is a fan-shaped mushroom with wavy margin and colored concentric zones (Fig. 1). *C. versicolor* is an obligate aerobe that is commonly found year-round on dead logs, stumps, tree trunks, and branches. The fungus occurs throughout the wooded temperate zones of Asia, Europe, and North America and may be the most common shelf fungus in the Northern Hemisphere. The mushroom belongs to the family Basidiomycotina.

Many different names have been used in the literature for *C. versicolor*, including *Agaricus versicolor*, *Boletus versicolor*, *Polyporus versicolor*, *Polystictus versicolor*, *Poria versicolor*, *Trametes versicolor*, *Yun-Zhi* (Chinese), and *Kawaratake* (Japanese). In North America, *C. versicolor* is commonly known as "turkey tail" mushroom. The morphological characteristics of *C. versicolor* fruiting body have been described (Soothill and Fairhurst, 1977) as follows: 3–5 cm across brackets that are semicircular, flattened, thin, and tough. Young brackets are flexible. Brackets usually occur in tiers and spread along branches. The upper surface is velvety and attractively marked with concentric zones of varying colors: brown, yellow, gray, greenish, or black. The margin is usually wavy. The mushroom has white spores that are oblong and cylindrical ($4–6 \times 2–2.5$ μm). In agitated

Fig. 1. *C. versicolor* mushroom (fruiting body) growing on tree trunk (courtesy of Frank L. Hoffman: www.all-creatures.org).

submerged culture, the fruiting body and spores do not form, and the fungus grows as dispersed or pelleted mycelium. More than 120 strains of *C. versicolor* have been recorded. Commercial PSK and PSP are obtained from the mycelium of CM-101 and Cov-1 strains of the fungus, respectively.

3. Composition and physical properties of *C. versicolor* polysaccharopeptides

PSP and PSK are light or dark brown powders that are soluble and stable in hot water. The compounds are polysaccharopeptides (Ueno et al., 1980a,b; Hotta et al., 1981) that are odorless and tasteless. The compounds do not have a definite melting point. Heating to more than about 120 °C gradually chars the polysaccharopeptides. The PSP/PSK polymers are soluble in water but insoluble in methanol, pyridine, chloroform, benzene, and hexane. An aqueous solution of PSP (1 g/100 ml water) is neutral, with a pH value of between 6.6 and 7.2. The $[\alpha]_D^{25}$ value of the PSP solution is in the range of 0–30° (Hotta et al., 1981).

Elemental analysis of PSK polysaccharopeptides reveals the following approximate composition: oxygen 47.5%, carbon 40.5%, hydrogen 6.2%, and nitrogen 5.2% (Ueno et al., 1980a). The powdered extract typically contains 34–35% soluble carbohydrate (91–93% β-glucan), 28–35% protein, ~ 7% moisture, 6–7% ash, and the remainder are free sugars and amino acids (Ueno et al., 1980a).

PSP and PSK are chemically similar and posses similar physiological activity profiles. PSK is a mixture of polysaccharides that are covalently linked to a number of proteins (Yang et al., 1992a). Polysaccharide and peptide moieties of PSP and PSK cannot be separated by native PAGE and chromatographic methods. Both products have a molar mass of approximately 100 kDa (Yang et al., 1992a; Ng, 1998) and their polypeptides contain large amounts of aspartic acid and glutamic acid (Ng, 1998). Acidic and neutral amino acids such as aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, and leucine account for 70% of all the 18 kinds of amino acids present (Hotta et al., 1981). *C. versicolor* polysaccharopeptides resist enzymatic proteolysis (Hotta et al., 1981).

PSP and PSK contain α-1,4 and β-1,3 glucosidic linkages in their polysaccharide moieties (Ng, 1998). D-glucose is the major monosaccharide present. Arabinose and rhamnose are the other principal monosaccharides in PSP. PSK contains fucose. Also present are galactose, mannose, and xylose (Wang et al., 1996b; Cheng et al., 1998). The polysaccharide moiety is highly branched.

4. Physiological activity of polysaccharopeptides

An extremely broad range of physiological effects has been linked with the use of *C. versicolor* polysaccharopeptides. Some of the main effects include the following: immunopotentiation by inducing production of interleukin-6, interferons, immunoglobulin-G, macrophages, and T-lymphocytes; counter immunosuppressive effects of chemotherapy, radiotherapy, and blood transfusion; antagonization of immunosuppression induced by tumors; inhibition of proliferation of various cancers by inducing production of superoxide dismutase (SOD), glutathione peroxidase, and general immune

enhancement; improvement of appetite and liver function; calming of the central nervous system; and enhancement of pain threshold. In addition, *C. versicolor* polysaccharopeptides can remedy intestinal disorders and are beneficial in the therapy of opportunistic microbial infections that suppress the immune response.

Polysaccharopeptides may benefit general health by inducing enzymes that mop up free radicals and mitigate oxidative damage. Current uses, administration, potential drug interactions, and contraindications of aqueous extracts of *C. versicolor* have been reviewed elsewhere (Chu et al., 2002). Both extracellular and intracellular polysaccharopeptides potentiate the immune system (Wang et al., 1996b). PSP-promoted proliferation of T-lymphocytes and the consequent immunopotentiation have been claimed to involve mediobasal hypothalamus in rats (Yu et al., 1996). The exact mechanisms of action of the polysaccharopeptides remain to be fully understood.

The polysaccharopeptides are recognized as biological response modifiers that are useful adjuncts to conventional therapy. In animal studies, PSK is effective when taken orally, intravenously, or intraperitoneally (Yang et al., 1992a). In human therapy, *C. versicolor* polysaccharopeptides are generally administered orally. Accumulating evidence suggests that the polysaccharopeptides are nontoxic even when administered at several times the therapeutically effective dosage and over extended periods. Extended use of PSP at 100-fold the normal clinical dose has not induced acute and chronic toxicity in animals. Polysaccharopeptides appear to be safe during pregnancy. No adverse effects of PSP have been observed in female reproductive and embryonic development in mice (Ng and Chan, 1997). PSP is not teratogenic. The use of *C. versicolor* polysaccharopeptides appears to be contraindicated when immune suppression is desired. Polysaccharopeptides reduce the potency of immunosuppressants such as cyclosporin. PSP administration partly restored immunosuppression induced by cyclophosphamide in rats (Qian et al., 1997).

PSP/PSK posses anticancer activity (Cho et al., 1988; Sakagami et al., 1991; Yang et al., 1992a; Dong et al., 1996; Mao and Gridley, 1998; Mao et al., 1996). Oral administration of PSK/PSP has controlled various carcinomas in experimental animals and humans (Ng, 1998). PSP is active against Ehrlich ascites carcinoma, P388 leukemia, and sarcoma 180 (Yang et al., 1992a). Although the polysaccharopeptides suppress proliferation of some human cancer cell lines (Yang et al., 1992a; Ng, 1998), not all cancers seem to respond to *C. versicolor* polysaccharopeptides (Wang et al., 1996b; Dong et al., 1997).

In vitro studies reveal that PSP acts selectively on HL-60 leukemic cells, arresting the cell in the G-phase of the cell cycle and inducing apoptosis (Hsieh et al., 2002). Normal lymphocytes are not affected by PSP (Hsieh et al., 2002). Other work (Dong et al., 1997) has not found antiproliferative effects of *C. versicolor* polysaccharopeptides against HL-60 human leukemic cells. PSK dose-dependently inhibited DNA synthesis in MCF-7, a human breast cancer cell line (Aoyagi et al., 1997). A PSK dose of 200 μg/ml caused a 50% inhibition of DNA synthesis (Aoyagi et al., 1997). *C. versicolor* polysaccharopeptides are useful in the complementary treatment of gastric (Nakazato et al., 1994) and other intestinal cancers.

Dong et al. (1996) observed that *C. versicolor* polysaccharopeptides dose-dependently inhibited the proliferation of a human hepatoma cell line (HEPG2), but not the normal human fetal hepatocytes. In nude mice, the progression of sarcoma 180 was measurably reduced by the administration of polysaccharopeptides (Dong et al., 1996). Data obtained

in vitro and in vivo suggest that PSP can slow the progression of murine H238 tumors (Mao et al., 1996). PSP has been observed to enhance the transcription of tumor necrosis factor gene in mouse peritoneal macrophages, indicating an immunomodulatory effect of PSP (Liu et al., 1993).

The anticancer activity of polysaccharopeptides is not caused by a direct cytotoxic effect. *C. versicolor* polysaccharopeptides are obvious immunoenhancers that potentiate the immune system in multiple ways (Chen et al., 1986; Sakagami et al., 1991; Yang et al., 1992a; Liu et al., 1993; Wang et al., 1996b; Qian et al., 1997; Ooi and Liu, 1999; Chu et al., 2002). This partly explains the antitumor activity. The enzyme SOD counters the tissue-damaging effects of free radicals. Intraperitoneal administration of *C. versicolor* polysaccharopeptides increased the SOD activity in the lymphocytes and thymus of normal mice. Similar enhancements in SOD activity and suppression of tumors were observed in tumor-bearing mice administered *C. versicolor* polysaccharopeptides (Wei et al., 1996). The SOD activity of in vitro-cultured cancer cell lines was enhanced by the presence of protein-bound polysaccharides, leading to suppression of cancer cell growth (Kobayashi et al., 1994a,b).

The in vitro anticancer activity of cisplatin was augmented by the presence of *C. versicolor* polysaccharopeptides (Kobayashi et al., 1994c). This effect was associated with the SOD-mimicking activity of the polysaccharopeptides. The presence of *C. versicolor* polysaccharopeptides reduced the cytotoxicity of cisplatin toward healthy cells (Kobayashi et al., 1994c). Yang and Chen (1998) and Yang et al. (1992b) purified a 10- to 16-kDa peptide from a crude extract of *C. versicolor* polysaccharopeptides. This peptide had a stronger antitumor activity than PSK and PSP (Yang et al., 1992b) and had immunopotentiary effect (i.e., enhanced white blood cell count and IgG levels in mice) (Yang et al., 1992b). The peptide was cytotoxic to several tumor cell lines by in vivo assays in mice (Yang and Chen, 1998).

Glutathione peroxidases play an important role in the defense against oxidative injury (Pang et al., 2000). PSK induced glutathione peroxidase activity in mouse peritoneal macrophages (Pang et al., 2000). This effect was ascribed to transcriptional induction of expression of mRNA (Pang et al., 2000). Polysaccharopeptide treatment of mouse peritoneal macrophages enhanced the glutathione peroxidase activity of the cells and prevented the inhibition of respiratory burst by *tert*-butylhydroxide (Jun et al., 1993). In experiments with fruit flies (*Drosophila melanogaster*), the intake of *C. versicolor* polysaccharopeptides increased the frequency of mating and the lifespan of flies (Li et al., 1993). Compared to controls, the administration of *C. versicolor* polysaccharopeptides enhanced the recovery of mice following gamma-irradiation-induced spleen damage (Lin et al., 1996). PSP has shown analgesic activity (Ng and Chan, 1997; Gong et al., 1998).

Hepatic lesions and mortality in mice given intravenous injection of influenza virus were reduced by oral or peritoneal administration of intracellular polysaccharopeptides to the animals (Chen et al., 1986). Both intracellular and extracellular polysaccharopeptides induced the production of serum interferon (Chen et al., 1986). Compared with the intracellular polymers, the interferon-inducing activity of the extracellular polymers was stronger (Chen et al., 1986). In vitro studies suggest that PSP may be useful against HIV-1 infection (Collins and Ng, 1997).

5. Production of *C. versicolor* polysaccharopeptides

5.1. Fermentation

C. versicolor polysaccharopeptides are commercially extracted from mushrooms or mycelia cultivated on solid substrates (Yadav and Tripathi, 1991; Park et al., 1994) and mycelial biomass produced in submerged fermentations (Yoshikumi et al., 1978a; Ueno et al., 1980a,b; Chen et al., 1981; Zhou et al., 1994; Cheng et al., 1998; Wang et al., 1996b). The major clinically approved polysaccharopeptide preparations PSK and PSP are obtained from submerged cultured mycelial biomass. In some cases, a mycelial mat formed on the surface of a static liquid medium has been used to extract the active compounds (Hotta et al., 1981).

Generally, glucose or sucrose is used as a carbon source in submerged culture. Nitrogen sources used include peptone, yeast extract, yeast powder, peanut flour, soybean flour, and soy sauce (*shoyu*). Phosphates are used for buffering, and magnesium sulfate is commonly the only other inorganic nutrient used. Fermentations are carried out at 24–28 °C under highly aerobic conditions. The pH is usually not controlled and, during culture, the pH typically declines from about 6.5 to 2.5. Bioreactors or fermenters used are similar to those described for culturing filamentous microfungi such as *Penicillium chrysogenum* (Chisti and Moo-Young, 1991).

In a typical submerged culture (Wang et al., 1996b), the medium used contained (per liter of distilled water) the following components: 24 g of potato dextrose broth, 5 g of peptone, 0.46 g of KH_2PO_4, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, and 20 mg of vitamin B1. The medium had a pH value of 6.0. Batch cultures were carried out in shake flasks held on a rotary shaker (150 rpm) at 27 °C for 7 days. Yoshikumi et al. (1978a) used a 50-l stirred fermentor. The medium consisted of glucose and yeast extract. Additional medium was fed stepwise during the 7-day culture. The final concentration of the biomass was $\sim$ 23 kg/m^3. The fermentation was carried out at 25 °C. We have attained biomass concentrations of up to $\sim$ 18 kg/m^3 in agitated submerged fermentations in various media (Cui, 2002). The total (intracellular and extracellular) polysaccharopeptides concentration after $\sim$ 7 days of culture was $\sim$ 4 kg/m^3 (Cui, 2002).

In submerged culture, *C. versicolor* can be grown as mycelial pellets or predominantly dispersed mycelium. Conditions favoring one morphology over the other are unclear, but hydrodynamic shear forces appear to play a role (Chisti, 1999a). Whether pelleted growth or dispersed mycelium is the preferred source of the bioactive polysaccharopeptides has not been established. Mycelial broths of *C. versicolor* tend to be highly viscous because of the suspended filamentous biomass and the extracellular dissolved polymers. Broths possess a yield stress (Zhou et al., 1994). Zhou et al. (1994) have discussed the factors that influence broth rheology and have provided quantitative rheological data.

Hotta et al. (1981) cultured the mycelium on the surface of a static nutrient broth. The broth (30 ml) was placed in an Erlenmeyer flask (200 ml). The culture lasted for 10 days at 25–27 °C. The nutrient medium contained 5% (g/100 ml) glucose, 0.2% peptone, 0.3% yeast extract, 0.1% KH_2PO_4, and 0.1% $MgSO_4 \cdot 7H_2O$. The mycelial mat that formed on the surface of the broth was collected and homogenized in physiological saline to inoculate larger (1-l) Erlenmeyer flasks containing 200 ml of nutrient medium. After

25 days of surface growth, only 4.3 g of biomass was obtained per flask. Clearly, therefore, surface culture on a static liquid medium is not satisfactory for commercial production of *C. versicolor* polysaccharopeptides.

In nature, *C. versicolor* grows on solid substrates and not in liquid media. Solid-state culture of *C. versicolor* is used in bioremediation applications, but only a few reports of this method of cultivation for producing the polysaccharopeptides exist. In one case, Yadav and Tripathi (1991) used wheat bran and wheat straw to culture the fungus. The solid substrates were supplemented with water, 1.0% (g/100 ml) superphosphate, and 1.5% urea. The optimal fermentation conditions were 55% moisture, pH 5.5, and 30 °C over 21 days of culture (Yadav and Tripathi, 1991).

Inert solids (zeolite and orchid soil) moistened with solutions of carbon (glucose, sucrose, and starch) and nitrogen sources have been used to grow the fungus (Park et al., 1994). Although the technology for large-scale production of food mushrooms is well established (Flegg et al., 1985), this type of culture is poorly controlled, labor-intensive, and prone to contamination. Methods for controlled solid-state fermentation have become available (Chisti, 1999b), but submerged culture appears to be the preferred option for producing *C. versicolor* polysaccharopeptides.

5.2. Recovery of polysaccharopeptides

Most commercial preparations of polysaccharopeptides use only the intracellular polymers recovered from the mushroom or submerged culture mycelium. The mushroom contains approximately 59% total polysaccharopeptides by weight. The mycelial biomass contains less at $\sim$ 30% of dry weight. The composition of the polysaccharopeptide product appears to depend on the source material and the method of recovery used.

A multistep hot water extraction of *C. versicolor* biomass appears to be necessary to recover the active polymers in sufficient amounts for use in modern commercial preparations. Typically, the biomass is extracted repeatedly with hot water and the combined extract is concentrated by evaporation under vacuum or ultrafiltration. The concentrate is subjected to fractional precipitation using ammonium sulfate or alcohol. Precipitates are redissolved, dialyzed, and may be further purified by chromatographic methods. The solution of the purified product is concentrated and spray-dried. Removal of low-molecular-weight substances appears to be important because these substances do not contribute to physiological activity and impart a bitter taste and disagreeable odor to the final product.

A typical extraction would use 2 kg of biomass (dry weight) in 30 l of water for the first stage. Next, two extractions of the filtered biomass residue will use less water (e.g., 20 l per step). Each extraction would be for 2–3 h. Polysaccharopeptides extraction yield may be increased possibly by disrupting the mycelial biomass in a bead mill (Chisti and Moo-Young, 1986) prior to, or during, extraction with hot water. The crude product extracted from the milled biomass is likely to contain many intercellular low-molecular-weight contaminants, but these are expected to be readily removable by the purification methods that are already used in PSP/PSK production trains.

In a typical processing scheme, Hotta et al. (1981) recovered the polysaccharopeptides by agitating the filtered mycelium of static liquid culture with distilled water at 98 $\pm$ 2 °C.

The extraction period was 3 h. The biomass slurry was cooled and filtered. The solid residue was further extracted as above. The extracts were combined and concentrated by evaporation under vacuum. The concentrated solution was saturated with ammonium sulfate to precipitate the polysaccharopeptides. The precipitate was dissolved in water and desalted by dialysis using a cellulose acetate membrane. The polysaccharopeptide solution thus obtained was concentrated to 5% of its original volume. Further ammonium sulfate precipitation steps followed. The final precipitate was desalted, dissolved in water, and purified by DEAE cellulose chromatography. One further ammonium sulfate fractionation step was used, and then the desalted precipitate solution was concentrated and spray-dried.

Ueno et al. (1980a,b) used sequential extraction of the mycelial biomass with aqueous alkaline solutions (0.1–1.0 M sodium hydroxide) at 90–95 °C. Extraction periods were

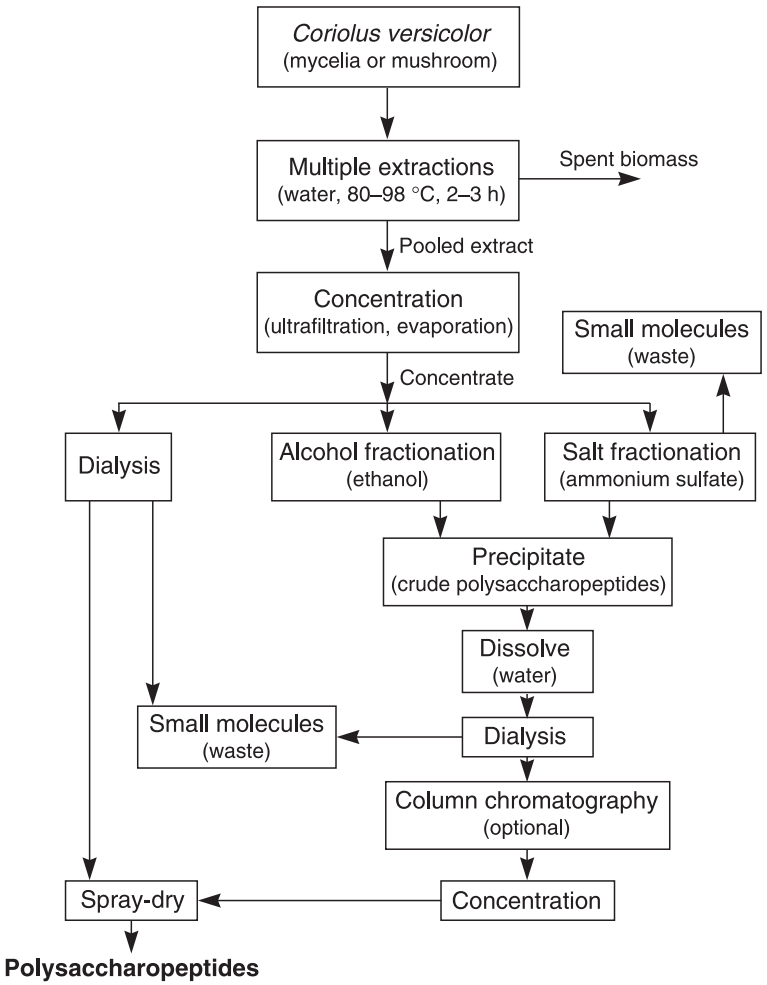

Fig. 2. Recovery and purification options for *C. versicolor* polysaccharopeptides.

different for different concentrations of alkali. Treatment with >2 M alkali was not recommended as it could degrade the bioactive polysaccharopeptides (Ueno et al., 1980a,b). The extracts were neutralized with mineral acid, pooled, and concentrated by ultrafiltration or reverse osmosis to remove the low-molecular-weight contaminants. The concentrated solution of polysaccharopeptides was spray-dried or freeze-dried to yield a brown powder. The process patents (Ueno et al., 1980a,b) specify extraction temperature and times of 80–90 °C and 20–600 min, respectively. Similar recovery methods have been used by others (Hotta et al., 1977; Wada et al., 1977; Yoshikumi et al., 1978b; Sugiura et al., 1974, 1980; Wang et al., 1996b).

In one case, the biomass was effectively extracted with hot aqueous solutions containing a surfactant (2% Triton X-100) (Park et al., 1992). Atomachi (1988) treated the mycelial extract with aqueous lead acetate. The precipitate produced was dissolved in water, and lead was removed by precipitating with sulfuric acid or hydrogen sulfide. The filtrate was treated with water-miscible organic solvents to recover the polysaccharopeptides. Ohara et al. (1997) extracted the mycelia and mushrooms by oxidation with HIO_4 or its salts, and by treatment with reducing agents. This procedure caused a mild hydrolysis of the product under acidic conditions.

Polysaccharopeptides are commonly recovered by precipitation from the concentrated extract. Ethanol precipitation (Sugiura et al., 1980; Chen et al., 1981; Kim et al., 2001) and ammonium sulfate fractionation (Hotta et al., 1981) are used frequently. A few studies have used HPLC in the final recovery stages to purify PSP (Yang and Chen, 1998), but this is generally impractical in large-scale processing. Conventional ion exchange chromatography on DEAE Sephadex and DEAE cellulose has been used effectively (Hotta et al., 1981; Park et al., 1992).

The major process alternatives for recovering *C. versicolor* bioactive polysaccharopeptides are summarized in Fig. 2. As with any commercial production process, the number of individual steps in the product recovery train should be kept to a minimum (Chisti, 1998). Thus, the precipitation and dialysis steps should be repeated a minimum number of times and no more than one chromatography step is recommended for the product intended for oral consumption. Because of the expense, spray drying of the final solution is preferable over freeze drying.

6. Concluding remarks

The protein-bound polysaccharides of *C. versicolor* have been used as immunopotentiators and therapeutics for years. In view of the clearly documented physiological benefits of these compounds, extensive research is underway on the structure, composition, production methods, and use of new *C. versicolor* strains for producing the therapeutic biopolymers.

Physiologically active polysaccharopeptides can be produced from *C. versicolor* mushrooms harvested in the wild or cultivated commercially. Furthermore, mycelial growth of *C. versicolor* in submerged fermentation provides a proven and scaleable method for commercial production of the polymers. The polymers produced in submerged culture can be extracted from the mycelial biomass and the biomass-free culture broth. The

polysaccharopeptides isolated from different sources (mushroom, mycelium, and biomass-free broth) differ somewhat in structure, composition, and physiological activity. Although *C. versicolor* is well suited to submerged culture, many commercial preparations of its polysaccharopeptides are sourced from the fruiting body.

Submerged batch culture of *C. versicolor* requires a well-balanced medium, sufficient aeration, and an initial broth pH of 5.5 ± 0.5. The optimal culture temperature is about 26 °C. The fermentation lasts about 5–7 days. The polysaccharopeptides are heat-stable and are easily recovered by extraction of the biomass (mushrooms or mycelia) with hot water or dilute alkaline solutions. The extract is concentrated and the active polymer is precipitated by salting out or organic solvents. Further purification is effected by dialysis, ultrafiltration, and chromatographic methods. The purified polysaccharopeptides are made into a powder by lyophilization or spray drying.

C. versicolor polysaccharopeptides can be a useful adjunct to conventional therapy of cancer and other diseases. Further work is necessary to establish the mechanisms of action and in vivo absorption of these compounds. Clinical trials are needed to prove some of the effects that have been observed in vitro and in experimental animals. Not every cancer appears to respond to *C. versicolor* polysaccharopeptides and in vivo studies are needed to clearly identify the applications where the administration of polymers will provide distinct benefits. *C. versicolor* polysaccharopeptides appear to be nontoxic in prolonged use and are claimed to benefit general health.

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