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Research paper

Utilization of corn fiber for production of schizophyllan

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ABSTRACT

Corn fiber is an abundant lignocellulosic biomass resource produced during the wet milling of corn. Although corn fiber is recalcitrant to enzymatic digestion, the fungus *Schizophyllum commune* was able to directly utilize corn fiber for production of the valuable bioproduct schizophyllan. Schizophyllan is a biopolymer composed entirely of glucose, with a β-1,3-linked backbone and single β-1,6-linked glucose side chains at every third residue. Schizophyllan is being developed for bulk biomaterial applications, such as in enhanced oil recovery and as a component of biolubricants. *S. commune* strain ATCC 38548 produced up to 6.8 ± 0.2 g dm^{-3} schizophyllan when grown in malt extract medium containing 10 g dm^{-3} untreated corn fiber in place of glucose. Pretreatment of corn fiber with alkaline hydrogen peroxide enhanced yields of schizophyllan. Corn steep liquor at 50 cm^3 dm^{-3} could replace malt extract as a nitrogen source, producing up to 5.4 ± 1.6 g dm^{-3} schizophyllan and 6.0 ± 0.5 g dm^{-3} schizophyllan from 10 g dm^{-3} untreated and pretreated corn fiber, respectively. Glucose (from corn syrup) further enhanced yields, substituting for the maltose component of malt extract. Schizophyllan produced from corn fiber exhibited a high molecular weight of 3.2 × 10^7 Da, with solution viscosity properties characteristic of schizophyllan. Utilization of corn fiber could reduce the cost of schizophyllan production and provide a value-added coproduct from corn processing biomass.

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1. Introduction

Corn fiber is an abundant lignocellulosic biomass coproduct of the corn wet milling process, primarily composed of the outer seed coat (pericarp) fraction of the kernel along with a variable amount of adherent starch [1]. Corn fiber is a convenient source of agricultural biomass, in that it is produced on site in the wet milling facility and does not require collection from fields. Conventionally, corn fiber is combined with steep liquor and/or stillage residues to form corn gluten feed, primarily used in cattle feed [2].

Compositional analyses of corn fiber vary considerably depending on the source of material and analytical methods. Generally, corn fiber has been reported to include on a mass fraction basis 30–50% arabinoxylan and 15–20% cellulose [3]. Adherent starch levels vary by production facility and on a daily basis, but can be 10–25% or greater [3]. Corn fiber is thus a rich potential source of fermentable sugars. However, corn fiber arabinoxylan is considered to be particularly recalcitrant to enzymatic saccharification, presumably because it is more highly derivatized and cross linked than that of corn stover and other grasses [4,5]. Nevertheless, some fungi, such as *Schizophyllum commune*, may be able to directly utilize corn fiber for production of valuable bioproducts.

S. commune is a white-rot fungus and ubiquitous mushroom that produces the extracellular polysaccharide schizophyllan. Schizophyllan is a homoglucan with a β-1,3-linked backbone and single β-1,6-linked glucose side chains at every third residue (Fig. 1) [6,7]. In humans, schizophyllan acts as a biological response modifier and a non-specific stimulator of the immune system. Schizophyllan is used commercially in vaccines, anti-cancer therapies, and as a bioactive cosmetics ingredient. However, its unique physical properties of high viscosity, film formation, and thermal stability suggest that it may be suitable for bulk biomaterials applications [8,9]. Schizophyllan is currently being tested by Wintershall Holding GmbH (Kassel, Germany) for use in enhanced oil recovery (www.wintershall.com). Schizophyllan solutions also showed promise as a component of biolubricants in friction and wear tests and by dynamic surface and interfacial tension measurements [10].

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[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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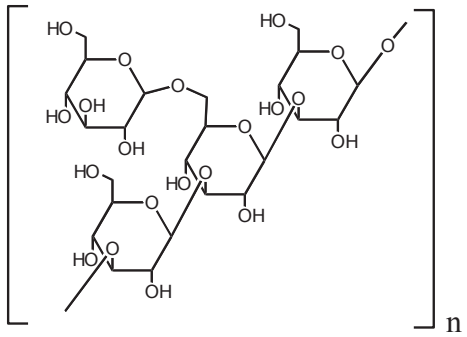

Schizophyllan repeat unit

Fig. 1. Chemical structure of the schizophyllan repeat unit (courtesy of Dr. Neil P.J. Price).

Schizophyllan could be particularly useful in biolubricants for metalworking, where aqueous solubility and thermal stability are important [11]. Currently, schizophyllan is produced from glucose and available only in small quantities of expensive purified pharmaceutical- and cosmetic-grade materials. Bulk biomaterials applications will call for less expensive production methods. The utilization of inexpensive agricultural biomass resources could reduce the cost of schizophyllan production.

Although glucose is used in conventional production of schizophyllan, *S. commune* can utilize a number of sugars and soluble starch for polysaccharide production [12]. *S. commune* produces biomass degrading enzymes, including xylanases and cellulases, and can directly utilize biomass resources [13,14]. However, several studies reported only low levels of productivity from biomass (<0.05 g g^{-1}) [15–17]. Recently, we demonstrated the feasibility of utilizing various agricultural biomass substrates, including corn fiber, for the production of schizophyllan in good yields [18]. The objective of the current study was to optimize production of schizophyllan from corn fiber, and replace expensive rich medium components with corn-based substrates.

2. Material and methods

2.1. Strain and culture conditions

Schizophyllum commune strain ATCC 38548 was grown on malt extract (ME) agar plates (20 g dm^{-3} malt extract, 1.0 g dm^{-3} peptone, 20 g dm^{-3} glucose, and 25 g dm^{-3} agar) at 28° C for 7–10 days. An approximately 7 mm × 7 mm square of mycelia was used to inoculate 250 cm^3 of the same medium without agar in a 500 cm^3 fluted Erlenmeyer flask with three 5 mm glass beads. This preinoculum culture was incubated in an orbital shaker at 4 s^{-1} (2.5 cm displacement) for 4–5 days at 30 °C. Cultures were grown in 150 cm^3 of ME basal medium (without glucose) in 500 cm^3 flasks, containing 10 g dm^{-3} untreated or pretreated corn fiber. Corn fiber was the kind gift of Aventine Renewable Energy, Inc., Pekin, IL, USA (now merged with Pacific Ethanol, Inc). Yellow dent corn (*Zea mays* var. *indentata*) was commercially grown in the vicinity of Tremont, Illinois (40° 52′ N, 89° 49′ W). Corn was harvested by combine and clean corn kernels were sold to a local grain cooperative (Tremont, Illinois) and transported from there to the wet milling facility (Aventine Renewable Energy, 40° 57′ N, 89° 64′ W). Corn fiber is a direct product of the wet milling process, composed primarily of the seed pericarp along with adherent starch. Corn fiber samples were collected by us immediately after milling at Aventine Renewable Energy on Sept. 8, 2011 and stored in our laboratory at −20 °C until use. Corn fiber was baked to dryness and ground in an IKA model A 11 analytical mill. Corn fiber pretreatment was with alkaline hydrogen peroxide as previously described [4]. Cultures were inoculated with 1.5 cm^3 of preinoculum and incubated in an orbital shaker at 4 s^{-1} (2.5 cm displacement) for 8 days at 30 °C. Alternatively, cultures were similarly grown in medium containing 0–20 g dm^{-3} untreated or pretreated corn fiber and 0–70 cm^3 dm^{-3} corn steep liquor. Certain cultures also were amended with 20 cm^3 dm^{-3} corn syrup. Bioreactor cultures were 1.5 dm^3 cultures containing 10 g dm^{-3} untreated corn fiber in 50 cm^3 dm^{-3} corn steep liquor, in 2 dm^3 vessels (Biostat, Braun Biotech, Allentown, PA), inoculated with 10 cm^3 of preinoculum. Bioreactor conditions were as previously described [10], at 30 °C with an aeration rate of 1.2 dm^3 min^{-1} and agitation at 3.3 s^{-1}, with removable baffles installed.

2.2. Recovery of schizophyllan solutions

A simplified downstream processing method was used [10]. Whole culture suspensions were diluted with an equal volume of deionized water, homogenized (Power Gen 700, Fisher Scientific) for 20 s, and then centrifuged at 10,900×*g* for 1 h at 4 °C. Culture supernatants were collected and stored at 4 °C.

2.3. Characterization of schizophyllan yields, FTIR spectra, molecular weight, and viscosity

For schizophyllan yield determinations, an equal volume of 95% ethanol was added to schizophyllan solutions. After one hour at 4 °C, precipitates were collected by centrifugation at 10900×*g* for 1 h at 4 °C. Polysaccharide precipitates were air-dried overnight to reduce the ethanol content and then lyophilized for dry weight determinations.

FTIR spectra were recorded on a Perkin Elmer Frontier Spectrometer (Waltham, MA) with a spectral resolution of 4 cm^{-1}. Schizophyllan molecular weights were determined by size exclusion chromatography as previously described [19]. Briefly, approximately 10 mg of lyophilized polysaccharide were dissolved in 1 cm^3 of nanopure water. The sample solution was filtered through a 0.45 μm filter (Pall, Port Washington, NY), applied to a Shodex SB-806MHQ high performance size exclusion chromatography (HPSEC) column (Showa Denko, Tokyo, Japan) and eluted with 4.25 g dm^{-3} sodium nitrate at a flow rate of 0.5 cm^3 min^{-1}. The column was calibrated with a set of pullulan molecular weight standards (Showa Denko, Tokyo, Japan). Separations were monitored using a Shodex OR-1 optical rotation detector (Showa Denko). Schizophyllan solution viscosity was measured using a Brookfield LVTDV-1 digital viscometer as previously described [19]. Commercial schizophyllan (cosmetic grade) was purchased from European Technologies, Inc., Denver, CO.

3. Results and discussion

3.1. Production of schizophyllan from corn fiber

Previously, we demonstrated the feasibility of schizophyllan production from a variety of agricultural biomass substrates, including corn fiber, in a basal medium (ME) composed of 20 g dm^{-3} malt extract and 1.0 g dm^{-3} peptone [18]. Subsequently, we developed a simplified process for recovery of schizophyllan for biomaterial applications [10]. Using this method, we carried out a time course of schizophyllan production in ME with 10 g dm^{-3} corn fiber, either untreated or pretreated with alkaline hydrogen peroxide (Fig. 2). Maximal schizophyllan yields from untreated and pretreated corn fiber were 6.8 ± 0.2 g dm^{-3} schizophyllan at day 8