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New production process of the antifungal chaetoglobosin A using cornstalks

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ABSTRACT

Chaetoglobosin A is an antibacterial compound produced by *Chaetomium globosum*, with potential application as a biopesticide and cancer treatment drug. The aim of this study was to evaluate the feasibility of utilizing cornstalks to produce chaetoglobosin A by *C. globosum* W7 in solid-batch fermentation and to determine an optimal method for purification of the products. The output of chaetoglobosin A from the cornstalks was 0.34 mg/g, and its content in the crude extract was 4.80%. Purification conditions were optimized to increase the content of chaetoglobosin A in the crude extract, including the extract solvent, temperature, and pH value. The optimum process conditions were found to be acetone as the extractant, under room temperature, and at a pH value of 13. Under these conditions, a production process of the antifungal chaetoglobosin A was established, and the content reached 19.17%. Through further verification, cornstalks could replace crops for the production of chaetoglobosin A using this new production process. Moreover, the purified products showed great inhibition against *Rhizoctonia solani*, with chaetoglobosin A confirmed as the main effective constituent (IC_{50} = 3.88 μg/mL). Collectively, these results demonstrate the feasibility of using cornstalks to synthesize chaetoglobosin A and that the production process established in this study was effective.

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Introduction

Chaetoglobosins are a group of cytochalasins that exhibit strong cytotoxicity to various kinds of cells, including animal, plant, and microorganism cells. Chaetoglobosin A and B were the first members of this family discovered in 1973,[1] and since then over 40 analogs have been identified.[2] Chaetoglobosin A, which is biosynthesized mainly by *Chaetomium globosum*, is the most abundant member of this family[3] and displays various biological activities. Chaetoglobosin A shows highly toxic effects against human cancer cell lines, a murine leukemia cell line, and *Caenorhabditis elegans*,[3–5] and also shows phytotoxicity against alfalfa seedlings[6] as well as acute toxic effects against various types of microorganisms such as *Setosphaeria turcica*, *Rhizopus stolonifer*, and *Coniothyrium diplodiella*.[7–9] Despite these broad effects, development of an effective and economically feasible chaetoglobosin A

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production method remains challenging due to the low output by microorganisms and the high cost of the required culture substrates and purification process.

The only source of chaetoglobosin A is its biosynthesis by microorganisms. Therefore, several studies have been conducted with the goal of optimizing the type of culture substrates used to achieve higher yields, including oat, potato, malt extract, corn, molasses, rice, nut, and yeast extract-glucose.[10–14] The other important cost related to chaetoglobosin A production is the low initial content of the crude extract (below 5%), which increases the complexity and cost of production and purification.[8] Laboratory purification protocols usually rely on various combinations of thin-layer chromatography and Sephadex LH-20 columns to improve the product amount and content in samples before they are subjected to high-performance liquid chromatography (HPLC).[8,15] Although these steps may afford excellent results in terms of yield and purification, the current procedures for the recovery and purification of chaetoglobosins are still unsuitable for large-scale production.

A key step to developing a new economical production procedure is the choice of inexpensive substrates for the biosynthesis of chaetoglobosin A. *C. globosum* is a traditional antagonist that is widely used for biological control,[16] and has been confirmed to show excellent biodegradability of cellulose substrates[17] such as cornstalks. Cornstalk accounts for over 30% of the production of all cellulosic biomass in China, but is generally left in the fields after harvest or is even burnt[18]; thus, the vast availability of this resource shows its potential as a low-cost raw material for chaetoglobosin A production. Alternatively, use of a complex culture system is likely to introduce additional impurities, which could increase the difficulty and cost of purification. Therefore, this study was designed to demonstrate the feasibility of using cornstalks to replace crops as a culture substrate, which could reduce the cost of chaetoglobosin A production. Furthermore, the optimal conditions for obtaining a higher content of chaetoglobosin A in the crude extract were determined, including the optimal extractant, operation temperature, and pH value. After the above handling procedures, the biocontrol efficiency of the partly purified products against a pathogenic fungus was evaluated. Finally, the cost of the crude extract using cornstalks as a substrate was compared with that using crops as raw materials. Together, our study provides an effective method for the large-scale production of chaetoglobosin A with high content and effective antibacterial activity.

Materials and methods

Microorganisms and culture conditions

C. globosum W7 was obtained from the Microbial Genetic Engineering Lab of Harbin Institute of Technology and was found to have the capacity of chaetoglobosin A production in our previous study (unpublished data). The strain was preserved in the China General Microbiological Culture Collection Center under accession number CGMCC 3.14974. Fermentation was carried out in 250-mL flasks with 10 g of cornstalks, 0.5 g ammonium chloride, and 20 mL water by spreading 1-mL suspensions containing 10^7 spores/mL of *C. globosum*. The strain was incubated at 28 °C for 14 days and then the surface of the medium was completely covered by spores. The fungus and medium were taken out from the flasks, tiled on glass plates, and air-dried at 28 °C for 24 h. Before use, the cornstalks (Dahe Agriculture LLC., Tsitsihar, China) were first smashed into particles with a size less than 0.25 mm (60 mesh per inch) using a plant pulverizer (Beijing light medical equipment Co. Ltd., Beijing, China). The cornstalks used in this study contained 41.725 ± 0.148% (m/m) carbon and 0.805 ± 0.035% (m/m) nitrogen, according to the results of the elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The medium consisted of cornstalks and ammonium chloride (nitrogen content 26.17%) with a ratio of 20/1 (w/w), so that the carbon/nitrogen ratio of the total medium was approximately 20/1.

Extraction effects of different solvents

The extraction effects of seven kinds of solvents were tested, including methanol, ethanol, ethyl acetate, acetone, dichloromethane, chloroform, and n-hexane. To reduce errors, ten flasks of fermentation residues were mixed and weighed after air-drying and smashing into particles with a size of less than 0.25 mm. Total smashed samples were averaged into 100 portions to ensure that each portion was fermented from about 1 g of cornstalk. The portions were then placed into 100-mL ground glass-stoppered flasks with the various organic solvents, respectively. Extraction was carried at room temperature (20–25 °C) for 24 h with 50 mL of organic solvent per flask, and this step was repeated twice.

After the extraction solvent and sample were separated with a G3 sintered glass funnel, the liquids were concentrated to about 2 mL using reduced pressure distillation (Shyarong Biochemical Instrument, Shanghai, China) under vacuum at 0.095 Pa, with final vacuum drying in Savant Speedvac (Thermal Technology LLC., Santa Rosa, CA, USA) at room temperature (20–25 °C). The distillation temperatures of different solvents were methanol at 50 °C, ethanol at 60 °C, ethyl acetate at 50 °C, acetone at 40 °C, dichloromethane at 40 °C, chloroform at 40 °C, and n-hexane at 60 °C. Dried extracts were redissolved in 1 mL acetone, and insoluble substances were removed via centrifugation at 15,000 × *g* for 10 min (Beckman Coulter Inc., Fullerton, CA, USA) before quantification of chaetoglobosin A with HPLC. After drying the solvent, the weights of the crude extracts were determined on a Precision Electronic balance (Sartious AG, Goettingen, Germany). All procedures were performed in triplicate independently.

Determination of optimal operating temperature

To determine the optimal temperature for chaetoglobosin A preparation, the crude extracts were dissolved into acetone with a final concentration of chaetoglobosin A of 1.0 mg/mL. Then, the Eppendorf tubes with 0.5 mL of the above solutions were placed at different temperatures (−20 °C, 0 °C, room temperature, 40 °C, 50 °C, 60 °C, 80 °C, 100 °C, 150 °C) for either 1 h or 24 h. Based on the results of the extraction effect test, acetone was chosen as the solvent. All procedures were

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