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Inhibition of HMG-CoA reductase by MFS, a purified extract from the fermentation of marine fungus *Fusarium solani* FG319, and optimization of MFS production using response surface methodology

ZHOU Yu[1], WU Wen-Hui[1, 2, 3#], ZHAO Qing-Bo[1], WANG Xiao-Yu[1], BAO Bin[1, 2*]

[1]*College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China;*

[2]*Shanghai Aquatic Products Processing and Storage Engineering Technology Research Center, Shanghai 201306, China;*

[3]*Institute of Marine Science, Shanghai Ocean University, Shanghai 201306, China*

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[ABSTRACT] The present study was designed to isolate and characterize a purified extract from *Fusarium solani* FG319, termed MFS (Metabolite of *Fusarium solani* FG319) that showed anti-atherosclerosis activity by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Response surface methodology (RSM) was employed to achieve an improved yield from the fermentation medium. The inhibiting effect of the isolate, MFS, on HMG-CoA reductase was greater than that of the positive control, lovastatin. The average recovery of MFS and the relative standard deviation (RSD) ranged between 99.75% to 101.18%, and 0.31% to 0.74%, respectively. The RSDs intra- and inter-assay of the three samples ranged from 0.288% to 2.438%, and from 0.934% to 2.383%, respectively. From the RSM, the concentration of inducer, cultivation time, and culture temperatures had significant effects on the MFS production, with the effect of inducer concentration being more pronounced that other factors. In conclusion, the optimal conditions for the MFS production were achieved using RSM and that MFS could be explored as an anti-atherosclerosis agent based on its ability to inhibit HMG-CoA reductase.

[KEY WORDS] Metabolite; HPLC analysis; HMG-CoA reductase; Response surface methodology

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Introduction

Marine fungi are an important source of novel chemical structures and secondary metabolites with potent biological activities [1-3]. Marine microorganisms possess unique physiological characteristics, such as salt tolerance, alkali tolerance, and anti-starvation, and special metabolic mechanisms for their defense system, because of their adverse living environments, such as oligotrophy, weak light, and low temperature [4]. The vast biodiversity of fungi found in the marine environment may be an important source of new pharmaceutical agents [5]. Thus, marine fungi have become a new resource for the discovery of bioactive substances in recent years. There are several reports on the development of marine compounds as new drugs [6-7]. Therefore, marine microorganisms continue to be a productive and successful focus of natural product research.

Until 2009, 530 species of marine fungi had been identified and classified into various species, including *Phoma*, *Macrophoma*, *Fusarium*, and *Aspergillus* [8]. The secondary metabolites of marine fungi have been shown to have a variety of bioactivities, such as antibacterial [9], antiviral [10], anti-tumor [11], and anti-cardiovascular effects [12]. *Fusarium solani* FG319 is a marine fungal strain, which can be found in the East China Sea [13].

Atherosclerosis is a major cause of ischemic heart disease and stroke, and one of the leading causes of mortality worldwide. It is widely appreciated that it represents a chronic inflammatory reaction of the artery wall in response to endo-

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[*Corresponding author] Tel: 86-15692165919, E-mail: bbao@shou.edu.cn

[#]Co-first author

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thelial distress, involving the inflammatory recruitment of leukocytes and the activation of resident vascular cells [14]. Cholesterol-containing lipoproteins are responsible for the induction of endothelial dysfunction and macrophage activation. Foam cell formation, which results from the uptake of cholesterol-containing lipoproteins by macrophages, is an essential step in the initiation and progression of atherosclerosis [15]. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes the conversion of HMG-CoA to mevalonate, a rate-determining step in the cholesterol biosynthesis pathway and also in the synthesis of non-steroidal isoprenoids that are essential for normal cell function [16]. HMG-CoA reductase inhibitors, such as statins, are currently the most powerful cholesterol-lowering drugs commercially available. It has been reported that the inhibition of HMG-CoA reductase by lovastatin demonstrates anti-arteriosclerosis activity by inhibiting cholesterol biosynthesis and lowering plasma cholesterol level in humans and animals [17-19].

Response surface methodology (RSM) is typically evaluated using a small number of standard synthetic test data and underan optimal experimental condition [20]. Xu *et al* [21] studied the extraction of taxol from fungus *Fusarium maire* by RSM with different medium component variable factors, demonstrating that, after optimization of the media, the yield of taxol was increased to 225.2 μg·L^{-1}. In the present study, single factor tests and the Box-Behnken method [22-23] were used to optimize the culture conditions for *Fusarium solani* FG319 to produce MFS (Metabolite of *Fusarium solani* FG319). RSM was employed to determine the effects of varying factors on the yield of MFS, including the concentration of the inducer (L-ornithine hydrochloride), the cultivation time, and the culture temperatures

The present study was also aimed at determining the inhibitory activity of the metabolite MFS from *Fusarium solani* FG319 on HMG-CoA reductase *in vitro*, and developing a direct and simple analytical high-performance liquid chromatography (HPLC) method for quantifying MFS in the fermentation medium of *Fusarium solani* FG319.

Materials and Methods

Experimental marine fungus

The marine fungus *Fusarium solani* FG319 was collected from seafloor sediments in the Chusan area (longitude 121°30′−123°25′; latitude 29°32′−31°04′) of the East China Sea. It was identified as a *Fusarium* species and preserved in College of Food Science and Technology, Shanghai Ocean University, Shanghai, China. The marine fungus *Fusarium solani* FG319 is not an endangered or protected species. Consequently, no specific permission was required for collection of the organism for the present study.

Culture media

The seed medium was consisted of 35 g glucose, 10 g starch soluble, 20 g soybean flour, 5 g bacteriological peptone, 5 g beef extract, 3 g yeast extract, 2 g sodium chloride, 0.5 g potassium phosphate dibasic, and 0.05 g magnesium sulfate per liter, at pH 5.8, and was sterilized at 121 °C for 15 min before use.

The fermentation medium (modified Czapek medium) was consisted of 2 g sodium nitrate, 1 g potassium phosphate dibasic, 0.5 g potassium chloride, 0.5 g magnesium sulfate, 0.01 g iron (II) sulfate, 30 g sucrose, and L-ornithine hydrochloride per liter and was sterilized at 121 °C for 15 min before use.

The incubation was carried out at 28 °C in an Erlenmeyer flask (500 mL) containing the seed medium (100 mL) at 160 r·min^{-1} for 3 d. Then the fermentation medium was inoculated with 1% seed culture.

ITS rDNA sequencing

We genotyped the strain using the ITS rDNA regions. ITS regions of the rRNA gene are often highly variable with respect to nucleotide composition and this characteristic can be used to distinguish morphologically distinct fungal species and strains of the same fungal species [24]. The ITS primers make use of conserved regions of 18S, 5.8S, and 28S rRNA genes to amplify the non-coding regions between them. ITS1 and ITS4 were used to amplify the different regions of internal transcribed spacer region [25].

Amplification was performed in a reaction volume of 25 μL containing of 50 ng·μL^{-1} of DNA, 1 mmol·L^{-1} each of dNTP, PCR reaction buffer (containing 1 mmol·L^{-1} $MgCl_2$), and 1U Taq polymerase together with 5 pmol of primers ITS1 and ITS4. All the components in the reaction except DNA were mixed together. Approximately 25 ng of the DNA was used as template. The PCR amplification cycle was consisted of 4 min at 94 ºC, followed by 30 cycles of 45 sec at 94 °C, 45 sec at 55 °C and 1 min at 72 °C, and with a final extension of 10 min at 72 ºC in an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany). PCR products were analyzed by electrophoresis at 70 V for 3 h in a 1.8% agarose gel in 1X Tris buffer and inspected under UV light in a transilluminator following ethidium bromide staining.

The PCR products were eluted using a SanPrep column DNA Agarose Gel Extraction Kit (SK8131), confirmed by fractionating on 1% agarose gel, and then directly sequenced using PCR primers as sequencing primers by Sangon Biotech Company, Shanghai, China.

BLAST analysis was carried out with the NCBI database. The BLAST analysis was performed with full length ITS sequences as queries to reveal relationships to published sequences. Highest homology and total score were noted for further analysis.

Extraction and quantification of the fungal metabolites

The marine fungus *Fusarium solani* FG319 was cultivated in the fermentation medium for 7 days at 22 °C, at a shaker speed of 160 r·min^{-1}. After fermentation, each culture was extracted with methanol overnight, and then sonicated at 400 W for 15 min. The combined extracts were filtered, and the su-

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