Vietnam Journal of Science and Technology ***56*** *(4A) (2018) 74-82*

EFFECT OF RICE BRAN OIL ON MYCELIAL BIOMASS PRODUCTION, BIOSYNTHESIS AND BIOACTIVITIES OF POLYSACCHARIDES BY *OPHIOCORDYCEPS SINENSIS* FUNGUS

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Received: 8 August 2018; Accepted for publication: 10 October 2018

ABSTRACT

Ophiocordyceps sinensis is an entomopathogenic fungus, which is one of the most valued natural remedies in Traditional Chinese Medicine. It continues to attract scientists' attention due tothe diversity of bioactive compounds such as polysaccharides, cordycepin, adenosine, andergosterol. Among them, exopolysaccharides (EPS) from the culture broth has possessedmany precious bioactivities, for example, immunomodulatory, antitumor, antioxidant activity. Interestingly, previous studies have demonstrated that plant oils have positive affects to the growth and biosynthesis of second darymetabolites of medicinal fungi. Therefore, the aim of this study is to enhance the biomass growth and EPS biosynthesis of *O. sinensis* fungus by implementing rice bran oil (RBO) at various concentrations from 1 % to 8 % (v/v) into the culture medium. The results illustrated that, in the RBO-added media, the biomass of mycelial and EPS production increased significantly compared to non-RBO medium. In particular, biomass yield was 26.6 g/L in medium within 6% RBO, and EPS production was 4.62 g/L in medium within 5 % RBO. Importantly, inhibition of xanthine oxidase activity of the IPS (2 % RBO) and EPS (8 % RBO) crudes rose considerably compared to the control. Besides, at 5 % of RBO, the anti-inflammatory activity of EPS was highest with IC_{50} = 824.947 (µg/ml). In addition, the minimum IC_{50} value of IPS was 1529.061 (µg/ml) when added to the culture medium 4 % RBO. In conclusion, these data have exposed evidence that the RBO was potential to dramatically exhibitthe biomass production and EPS biosynthesis of *O. sinensis* and improve their bioactivities.

Keywords: anti-inflammatory, *Ophiocordyceps sinensis*, polysaccharides, rice bran oil, xanthine oxidase.

1. INTRODUCTION

The Chinese caterpillar fungus, *Ophiocordycep ssinensis* (also known as *Cordyceps sinensis*) is an important traditional fungal drug that has been commonly used for hundreds of

years as a tonic and/or drug. Pharmacological studies on *O. sinensis* have revealed that the fungus has multiple biological and pharmacological effects, such as immunomodulatory, anti-inflammatory, antioxidant, anti-ageing, antitumour, neuroprotective, hepatoprotective and renoprotective effects [1]. Currently, the implementation of plant oils in the culture of medicinal mushrooms is being researching by scientists. The plant oils stimulate the growth of fungi and EPS production during the fermentation process [2]. In addition, the plant oil is known as an anti-foaming agent during fermentation. Hence, it stimulates the growth of fungi and secondary metabolism for several medicinal fungi [2, 3].

During the polishing process of the rice, a unique vegetable oil rich in antioxidants produced from the outer layer of rice is what we called Rice bran oil (RBO). RBO contains several compositionswhich would potentially providehealth care beneficial. Gamma-oryzanol, tocotrienol, tocopherol, squalene and other phytosterols in RBO high antioxidant property against free radicals, cancer, and enhance the immune system, nervous system and endocrine health. Therefore, in this study, we carry out the survey of the effects of RBO on mycelial biomass production, EPS biosynthesis of *O. sinensis* mushroom as well as the anti-inflammatory andinhibition of xanthine oxidase (XO) activity of polysaccharides [4].

2. MATERIALS AND METHODS

2.1. Material

Ophicordyceps sinensis strain was supplied by Dr. Truong Binh Nguyen (Dalat University, Da Lat, Lam Dong, Viet Nam). It was maintained on Potato Dextrose Agar (PDA) medium at 4 °C.

2.2. Methods

2.2.1. Investigation of the effects of plant oils on mycelial biomass and EPS production of Ophiocordyceps sinensis

Cultivation in liquid media was carried out in 500 mL plastic container containing 200 ml of: 200 g/L potato, 50 g/L saccharose, 6 g/L peptone, 4 g/L yeast extract, 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 0.5 g/L $CaCl_2$, 0.2 g/L $MgSO_4$, 1 % (v/v) Tween 80, 1–8 % bran rice oil. The mediums were incubated at 22 ^oC for 30 days.

2.2.2. Harvesting the mycelial biomass and extraction of intracellular polysaccharides

The assay was proceeded by following the method of [5] which was followed with minor modifications. The biomass was dried at 60 °C to constant mass. The dry weight of the biomass (g/L) was evaluated.

Twenty grams of biomass power was extracted with water at 60 ^oC proportion of 1:10 (w/v) for 3 hours. The supernatant was collected, concentrated and precipitated with 4 volumes of 96 % ethanol (v/v), stirred vigorously, and kept at 4 ^oC for 24 hours. The mixture was centrifuged (8000 rpm/10 minutes), the precipitated in tracellular polysaccharides (ISP) was dissolved in distilled water, and the ISP content was determined using phenol–sulfuric acid method.

2.2.3. Extraction of exopolysaccharides

The culture broth was received and then concentrated by a rotary vacuum evaporator. The exopolysaccharides (EPS) was isolated by precipitation with ethanol 96° in the ratio 1:4 (v/v) at 4 °C for 24 hours and centrifuged (6000 rpm, 20 min). Finally, the sample was lyophilized and stored at 4 °C. The polysaccharide content of EPS was determined by the phenol-sulfuric acid method [6].

2.2.4. In vitro xanthine oxidase assay

The inhibitory effects of polysaccharides on *in vitro* xanthine oxidase activity were determined using a spectrophotometric method [7]. 200 µl of the test sample was added to 60 µl of 50 mM phosphate buffer solution (pH 7.5). 20 µl of prepared enzyme solution (0.05 units/ml in 50 mM phosphate buffer, pH 7.5) was then added and the assay mixture was incubated at 25 ^{o}C for 15 min. Next the enzymatic reaction was initiated by the addition of 320 µl of 150 mM xanthine substrate and the mixture was incubated at 25 ^{o}C for 30 min. The reaction was terminated by the addition of 100 µl hydrochloric acid (1 N). The absorbance of the assay mixture was measured at 290 nm. Allopurinol, a known inhibitor of XO, was applied as a standard. The control solution was also prepared as above but without sample [7]. XO activity was expressed as the percentage inhibition of XO, calculated as: % inhibition = $((A_0-A_{b0}) - (A_1-A_{1b}))/(A_0-A_{b0}) \times 100$ %, where A_0 was the absorbance of the control, A_1 was the absorbance of the sample and A_b was the absorbance of the solution without XO.

2.2.5. Determination of in-vitro anti-inflammatory activity

Anti-inflammatory activity was performed according to the modified method of [8]. The reaction mixture was contained 1.0 mL of test sample of different concentrations, 2 mL of 25mM acetate buffer (pH 5.5), and 0.5 mL of 0.16 % (w/v) bovine serum albumin fraction (BSA). The mixture was incubated at 37^{o}C for 20 min and then heated at 67 ^{o}C for 3 min. The denaturation process is stopped by cooling the samples and finally the turbidity was measured by using spectrophotometer at 660 nm. Diclofenac sodium was used as standard drug and the control was taken without the extract. The denaturation of protein inhibition by the extract and standard were determined. Percentage of protein inhibition = % inhibition = $((A_0-A_{b0}) - (A_1-A_{1b}))/(A_0-A_{b0}) \times 100\%$, where A_0 was the absorbance of the control, A_1 was the absorbance of the sample and A_b was the absorbance of the solution without BSA.

2.2.6. Data analysis

The experiment was performed in triplicate. Each test was performed and then repeated three times. Data were presented as mean ± SD. Comparisons of the measurement data between multiple groups were performed with one-way ANOVA test. The statistical process was performed with SPSS 20.0 software.

3. RESULTS AND DISCUSSION

3.1. Effects of RBO on mycelial biomass production

The yield of biomass from *O. sinensis* in various RBO-containing media were showed in the Figure 1.

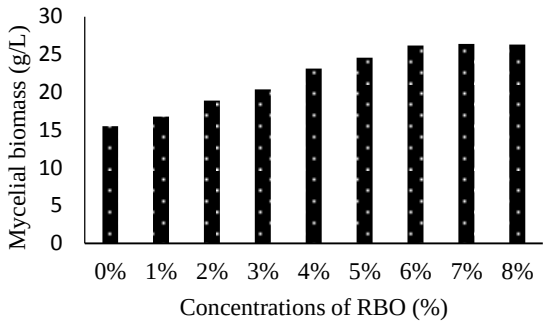

Figure 1. The biomass yields of *O. sinensis* fungus in different concentrations of RBO.

The chart above has exposed that RBO has significant influence to biomass production. When added RBO (1 - 8 %) to broth culture, the biomass yields were higher than control (15.5 g/L), 4 % of coconut oil (about 24.3 g/L) and 3 % of sunflower oil (26.4 g/L) (Figure 1) [9]. Particularly, the dried weight achieved the highest at 6 % of RBO (about 26.6 g/L). The content of linoleic acid and oleic acid in RBO accounted for about 34.4 %, 38.4 %, respectively [10]. It was a major ingredient related to stimulating the biomass production of fungi because it could enhance nutrient uptake in the culture medium [3, 11]. However, this mechanism remains unclear.

Therefore, RBO stimulated the fungal growth, which was similar to the report for culturing *G. frondosa* fungus [3]. Specifically, at 6 % of RBO, the biomass yield of *O. sinensis* was the highest.

3.2. Effects of RBO on EPS biosynthesis

After collecting the biomass, EPS crudes were isolated from different concentrations of RBO - containing media (1 – 8 %). It was a kind of extracellular polysaccharides with many vital bioactivities which was secreted in culture medium during the grown of fungi.

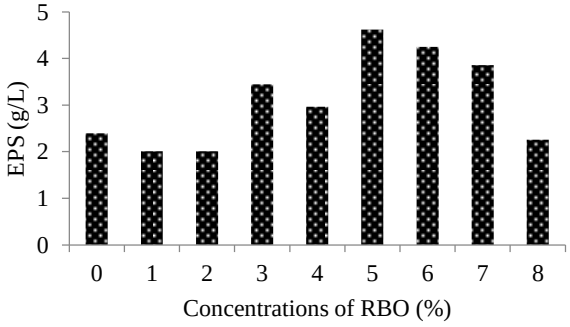

Figure 2. The EPS biosynthesis of *O. sinensis*fungus in different concentrations of RBO.

The EPS content of the control reached to 2.3 96 g/L. The Figure 2 for the medium with 1 %, 2 % RBO supplementing, EPS accumulation was lower than the control. However, the

amount of EPS was directly correlated with increasing concentration from 3-5 % and dropped at more than 5 %. In short, at the concentration of 5 %, the EPS was secreted the most (about 4.62 g/L), which was as twice as the control.

In conclusion, the results have demonstrated that RBO not only stimulated the growth of *O. sinensis* fungus, but also enhanced the EPS biosynthesis of the fungus. Thus, to improve the mycelial biomass production and EPS biosynthesis of *O. sinensis* fungus, it was suggested that the rice bran oil-containing medium of 5 % was an ideal condition of cultivation. Therefore, to improve the biomass fungus and the EPS of *O. sinensis*, it was suggested that addition of 5-6 % RBO was the most appropriate.

3.3. The polysaccharide content of EPS crudes

The polysaccharide content of EPS crudes was determined by phenol-sulfuric acid as showed in the Table 1.

Table 1. The polysaccharide content of IPS and EPS crudes.

Concentrations of RBO (%)	0	1	2	3	4	5	6	7	8
IPS (%)	59.78 ± 8.735	59.78 ± 4.245	60.33 ± 0.543	45.47 ± 0.830	62.14 ± 0.628	56.70 ± 1.131	53.99 ± 0.830	53.08 ± 0.314	46.56 ± 1.131
EPS (%)	22.37 ± 0.415	20.11 ± 0.471	17.30 ± 0.415	14.67 ± 0.272	15.49 ± 0.272	28.17 ± 0.157	45.29 ± 10.299	28.89 ± 3.287	21.83 ± 7.379

Table 1 shows that RBO does not significantly affect the polysaccharide content in IPS as well as in EPS. Specifically, for IPS crudes, the content of polysaccharide in the RBO supplementing medium (1 % - 8 %) was fluctuating from 45.47 to 62.14 %. Similarly, RBO did not have a clearly impact on the polysaccharides content in EPS crudes.

3.4. Xanthine oxidase inhibition activity of polysaccharides

Xanthine oxidase is an important enzyme in the metabolism of xanthine into uric acid. Previous studies showed that the inhibition of XO activity has the potential to falling uric acid levels in the blood and treating gout. Consequently, this study helped screen polysaccharides in RBO containing media have the XO inhibitory capacity. Results showed that the IC_{50} value of allopurinol, a clinical XO inhibitory drug,was 0.7 ± 0.01 µg/ml under the assay conditions.

In Table 2, IPS-2 extraction from biomass of *O. sinensis* cultured with 2 % RBO supplementation had the highest XO inhibitory activity (capable to inhibit 26.81 % at concentration of 200 µg/ml). The remaining IPS was slightly inhibit the XO enzyme.

Table 3 exhibited that EPS obtained from broth culture of *O. sisnesis* with RBO supplement at different concentration have provided different bioactivity. Particularly, EPS-8 yielded highest inhibitory activity (capable to inhibit 16.03 % at concentration of 500 µg/ml).

Table 2. Inhibition of xanthine oxidase (XO) activity of IPS (%).

Sample	Concentrations µg/ml					
	6.25	12.5	25	50	100	200
IPS-0	-	-	-	-	-	-
IPS-1	-	-	-	-	-	-
IPS-2	-	-	25.09	25.58	24.11	26.81
IPS-3	0.493	11.184	1.316	-	-	-
IPS-4	-	-	-	-	-	
IPS-5	-	-	-	-	-	
IPS-6	-	-	-	-	-	
IPS-7	1.434	1.195	-	-	-	-
IPS-8	0.149	-	-	-	-	-

IPS-0 – IPS-8: EPS harvest from difference RBO supplementing medium, "-": no activity.

Table 3. Inhibition of xanthine oxidase (XO) activity of EPS (%).

Sample	Concentrations (µg/ml)				
	31.25	62.5	125	250	500
EPS-0	-	-	-	-	-
EPS-1	-	-	-	-	-
EPS-2	-	6.48	4.37	-	-
EPS-3	4.61	-	-	-	-
EPS-4	4.91	0.85	-	-	-
EPS-5	3.56	-	-	-	-
EPS-6	-	-	-	-	-
EPS-7	-	-	-	-	-
EPS-8	1.35	1.57	6.05	12.84	16.03

EPS-0 – EPS-8: EPS harvest from difference RBO supplementing medium, "-": no activity.

XO inhibitory activity of *O. sinensis* has been reported previously. The result of [12] demonstrated a CPS-1 polysaccharides fragment extracted from *O. sinensis* was inhibiting XO with IC_{50} of 0.7 µg/ml. In Viet Nam, Huynh Thu (2017) also exposed similar results [7]. Despite our study has revealed bioactivity of IPS and EPS to XO-inhibition were lower than previous studies, but polysaccharide extract from fungi, which was cultivated in broth medium within RBO supplement also showed its own potential to inhibit XO.

3.5. Determination of *in-vitro* anti-inflammatory activity

Nowadays, in the method of treatment of gout, in addition to decreasing hyperuricemia, reduce both pain and inflammation around the joints was used. Thus, we investigated the anti-inflammatory activity of polysaccharides.

The denaturation of protein inhibition assay is a reliable method for assessing the potential of compounds or drugs with anti-inflammatory activity. Diclofenac, standard anti-inflammatory drug showed IC_{50} was 55.513 ± 10.303 µg/ml (Fig. 3). This showed that diclofenac was able to make a bond with protein to build a stable structure to protect the protein from heat denaturation.

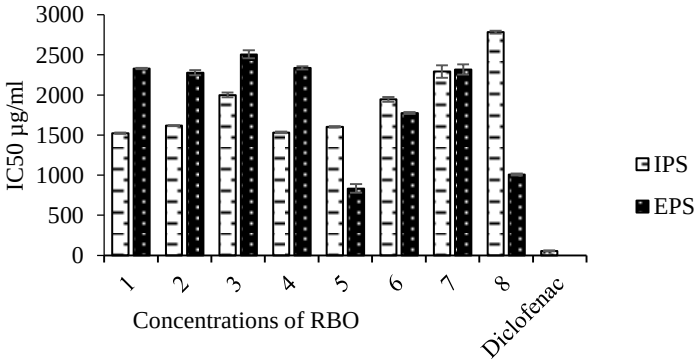

Figure 3. The anti-inflammatory activity of polysaccharides in different concentrations of RBO ($p < 0.05$).

The IC_{50} values of the polysaccharides exhibiting strong anti-inflammatory activities in vitro was present in Fig. 3. The result exhibited that EPS obtained from culture of *O. sinensis* with rice bran oil with different concentrations were able to inhibit BSA. In particular, high EPS obtained from culture medium supplemented with 5 % rice oil had the highest albumin inhibitory activity and had IC_{50} values of 824.947 ± 69.229 (µg/ml). In addition, the minimum IC_{50} value of IPS was 1,529.061 ± 4.676 (µg/ml) when added to the culture medium 4 % RBO.

When RBO was added to culture mediums, albumin inhibition activity of EPS1 - 8 samples and IPS1 - 8 were higher than that of control samples (IC_{50} > 3000 µg/ml). Thus, this suggests that RBO is stimulating the anti-inflammatory activity of polysaccharides. Some of component in the RBO as γ-oryzanol, acid hydroxycinnamic derivatives (HADs) showing XO inhibitory action [13, 14]. Similarly, polysaccharides obtained from *C. militaris* was demonstrated that has a strong anti-inflammatory activity [15]. These data strongly indicate that the *in vitro* effect of polysaccharides from *O. sinensis* is potential inhibition for the prevention and treatment of gout.

4. CONCLUSIONS

The study has indicated that RBO was an important composition for culturing *O. sinensis* fungus. RBO concentrations of 5 % and 6 % are the suitable concentrations for fungal growth and EPS biosynthesis. Although the ability to inhibit XO enzyme is not high, only IPS at 2 % RBO supplementation and EPS at 8 % have inhibitory activity. In addition, polysaccharides also stronger anti-inflammatory properties than the control in which the most IC_{50} of IPS at 4 % RBO containing medium was 1,529.061 µg/ml and the most IC_{50} of EPS at 5 % RBO containing medium was 824.947 µg/ml. In conclusion, it suggested that using the RBO to culture *O. sinensis* fungus is an important strategy because it stimulates not only the mycelial biomass production of the fungus and the EPS biosynthesis but also improves the in vitro anti-

inflammatory activities of EPS. Therefore, the polysaccharides from *O. sinenis* cultured in RBO containing mediumare candidates for the treatment of gout.

***Acknowledgements*.** We are grateful to Dr. Truong Binh Nguyen (Researcher at Dalat University, Vietnam) who have provided the *Ophiocordyceps sinensis* strain.

REFERENCES

1. Singh R., Negi P. and Dwivedi S. K. - *Ophiocordyceps sinensis*: The medicinal caterpillar mushroom, New Age Herbals, Springer (2018) 115-133.
2. Bolla K., Hima B.N., Samatha B. and Charya M. - Effect of plant oils, surfactants and organic acids on the production of mycelial biomass and exopolysaccharides of *Trametes* spp, International Journal of Agricultural Technology **7** (4) (2011) 957-965.
3. Hsieh C., Wang H. L., Chen C. C., Hsu T. H. and Tseng M. H. - Effect of plant oil and surfactant on the production of mycelial biomass and polysaccharides in submerged culture of *Grifola frondosa*, Biochemical Engineering Journal **38** (2) (2008) 198-205.
4. Liang Y., Gao Y., Lin Q., Luo F., Wu W., Lu Q. and Liu Y. - A review of the research progress on the bioactive ingredients and physiological activities of rice bran oil, European Food Research and Technology **238** (2) (2014) 169-176.
5. Wang L., Wang G., Zhang J., Zhang G., Jia L., Liu X., Deng P. and Fan K. - Extraction optimization and antioxidant activity of intracellular selenium polysaccharide by Cordyceps sinensis SU-02, Carbohydrate Polymers **86** (4) (2011) 1745-1750.
6. Kim H. and Yun J. - A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures, Journal of applied microbiology **99** (4) (2005) 728-738.
7. Huynh T., Vu A.T., Nguyen T.H., Dang N.H.C.and Dinh M.H.- Inhibition of xanthine oxidase activity of extracts from some medicinal mushrooms, VNU Journal of Science: Natural Sciences and Technology **33** (2S) (2017) 192-198.
8. Mizushima Y. and Kobayashi M. - Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, Journal of Pharmacy and Pharmacology **20** (3) (1968) 169-173.
9. Le T. T. H., Bach T. B. P., Nguyen T. T. T., Tran M. T., Huynh T., Nguyen T. T., and Dinh M. H. - Optimization of exopolysaccharide production in liquid culture of *Ophiocordyceps sinensis* fungus with addition of olive oil, VNU Journal of Science: Natural Sciences and Technology **33** (1S) (2017) 174-181.
10. Cicero A. and Gaddi A. - Rice bran oil and γ-oryzanol in the treatment of hyperlipoproteinaemias and other conditions, Phytotherapy Research **15** (4) (2001) 277-289.
11. Krishna A. G., Gaurav R., Singh B. A., Kumar P. P. and Preeti C. - Coconut oil: chemistry, production and its applications-a review, Indian Coconut Journal **53** (3) (2010) 15-27.
12. Li S. P., Zhao K. J., Ji Z. N., Song Z. H., Dong T. T., Lo C. K., Cheung J. K., Zhu S. Q. and Tsim K. W. - A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury, Life sciences **73** (19) (2003) 2503-2513.

13. Nagasaka R., Chotimarkorn C., Shafiqul I. M., Hori M., Ozaki H. and Ushio H. - Anti-inflammatory effects of hydroxycinnamic acid derivatives, Biochemical and Biophysical Research Communications **358** (2) (2007) 615-619.
14. Saenjum C., Chaiyasut C., Chansakaow S., Suttajit M. and Sirithunyalug B. - Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran, Journal of Medicinal Plants Research **6** (6) (2012) 1070-1077.
15. Lee J. S., Kwon D. S., Lee K. R., Park J. M., Ha S. J. and Hong E. K. - Mechanism of macrophage activation induced by polysaccharide from *Cordyceps militaris* culture broth, Carbohydrate polymers **120** (2015) 29-37.