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Optimization of Soy-based Media for the Production of Biologically Active Exopolysaccharides by Medicinal Mushroom *Trametes versicolor*

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Abstract

Background and Objective: Diabetes mellitus is the fifth leading cause of death in the world. Damaging effects of diabetes include advanced metabolic complications and various organ lesions. More than 90% of diabetic cases belong to type 2 diabetes. For decades, medicinal fungi such as *Trametes versicolor* have been considered as treatments for diabetes. The fungi exopolysaccharides show α-glucosidase activity in the intestinal membrane.

Material and Methods: In this study, Iranian *Trametes versicolor* was cultured in various media based on soy milk, cow milk and soy protein extraction. After selecting appropriate culture media for biomass and exopolysaccharide production, response surface method with Box-Behnken design was used to investigate effects of independent variables of glucose (g l^{-1}), soy milk (v v^{-1}) and pH on biomass and exopolysaccharide production and optimization of these products. An animal (rat) model of streptozotocin-induced diabetic rats was used to investigate effects of exopolysaccharides on diabetes. Rats were treated with exopolysaccharides for 21 days.

Results and Conclusion: Results showed that the culture media containing soy milk was appropriate for the growth and production of fungal products. Productions of biomass and exopolysaccharides increased to 2.87 g l^{-1} and 1.37 g l^{-1} respectively. Based on the analysis of response surface method, pH with *p*-value of 0.0004 and pH and soybean interaction with *p*-value of 0.0129 included significant effects on exopolysaccharide production. In optimum condition of the culture media with pH 4.67, 13.01 g l^{-1} glucose and 75% v v^{-1} soybean milk, biomass and exopolysaccharides reached 21.80 g l^{-1} and 9.6 g l^{-1}. In diabetic rat model treated with exopolysaccharides, a 50.38% decrease in blood glucose was seen while triglycerides, cholesterol and low-density lipoproteins included 89, 20 and 21.67%, respectively. Furthermore, high-density lipoproteins increased by nearly 5.12%.

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1. Introduction

Diabetes mellitus is the fifth leading cause of death in the world, with an increasing prevalence worldwide [1]. It is estimated that 366 million adults are affected by diabetes by 2030 [2]. Damaging effects of diabetes include advanced metabolic complications and various organ lesions such as retinopathy, neuropathy, cardiovascular diseases with symptoms such as hyperglycemia, excessive thirst, delayed healing of sores, increased urine glucose [1]. More than 90% of diabetic cases are linked to type 2 diabetes [3]. Type 2 diabetes may be delayed or eliminated by improving lifestyle. However, there are synthetic chemicals and biochemical drugs used to treat diabetes and control blood glucose. Drugs used to treat diabetes such as metformin, troglitazone and acarbose increase insulin secretion, improve cell access to glucose, decrease glucose release from the liver or limit absorption of carbohydrates from the intestine [4]. However, these drugs include harmful side effects such as kidney and liver injuries and weight gain [1]. Although insulin is the major treatment of type 2 diabetes, treatment of diabetes is still a big challenge and patients shift to herbal remedies. Diabetic patients may prefer traditional treatments for several reasons such as dissatisfaction with and side effects of modern drugs and verified benefits of medicinal herbs [5]. Of the herbs of traditional Chinese medicine, ginseng, garlic and cinnamon have been used for the treatment of diabetes [6].

In several countries, especially Japan, China, Korea and India, medicinal fungi have been recommended as natural sources of bioactive compounds for the treatment of diabetes. Glucose-lowering fungal metabolites include polysaccharides, proteins and several other biochemicals. Of these metabolites, polysaccharides specifically improve pancreatic function in decreasing blood glucose by increasing insulin releases from β-cells of the pancreas [7]. Medicinal fungi such as *Ganoderma lucidum*, *Ganoderma atrum*, *Grifola frondosa* and *Trametes* (*T.*) *Versicolor* decrease blood glucose through specific mechanisms [8]. For example, *T. gibbosa* decreases lipid production and hence decreases production of oxygen free radicals, preventing further damages to pancreatic cells and *Grifola frondosa* enhances synthesis of glycogen from HePG2 cells in the liver [9]. Consumption of fungi causes significant changes in cholesterol, triglycerides and low-density lipoproteins (LDL) as well as high-density lipoproteins (HDL) [10]. The *T. versicolor* is a basidiomycete fungus, which plays important roles in pharmaceutical industries due to the production of polysaccharides. This fungus is one of the most important medicinal fungi, which has been studied in many countries, especially in Japan and China. The fungal products include polysaccharide krestin and polysccharopeptide. Exopolysaccharides (EPS) of *T. versicolor* include large quantities of glucose and low quantities of galactose, mannose, arabinose and xylose. The molecular chain contains glycosidic bonds of β (1→3) and β (1→6). The fungus includes properties such as anti-tumor, antioxidant, immune regulator, antiviral, antibacterial and antidiabetic properties [11,12]. The active constituents of *T. versicolor* include (1→3)glucan, coriolin (I) and deoxycoriolic acid (II) [13]. Investigation of the compounds extracted from *T. versicolor* shows that they have inhibitory effects on α-glucosidase enzyme with various inhibitory potentials. The most inhibition effect has been seen in the composition of eps-F4-1, which is a polysaccharide with triterpenoid. Inhibition of this enzyme by polysaccharide is linked to α (1→4) glycosylated bonds in the polysaccharide structure and the proportion of D-glucose and D-galactose in the polysaccharide. The fungal polysaccharides inhibit α-glucosidase enzyme in the intestinal membrane, prevent decomposition of carbohydrates into sugar substitutes and prevent increases in blood glucose levels, especially after consumption of carbohydrates [14].

Several studies have been carried out to increase production of polysaccharides by *T. versicolor*. According to Tavares et al., culture media characteristics, especially glucose and pH, play important roles in increasing production of polysaccharides in *T. versicolor* [15]. Que et al. showed that with changing in pH, production of polysaccharides by *T. versicolor* increased significantly [16]. In 2007, researchers identified New Zealand Wr74 strain from *T. versicolor*. They used a low-cost medium based on milk for the production of biomass and EPS [17]. Wang et al. increased EPS content to 2.7 g l^{-1} and yielded 2.56 g l^{-1} using 0.8 mM of farnesol quorum sensing molecules. Farnesol affects morphology of the fungi, which facilitates EPS releases [18]. The researchers used 5 mM of tyrosol, which increased by 1.95-fold IPS and yielded 39.90 g l^{-1} [19]. The purpose of the present study was to increase biomass and EPS production by Iranian *T. versicolor* using alteration in compositions of the culture media and optimization of glucose, pH and soy milk at three levels. Furthermore, response surface method with Box-Behnken design was used. This method allows study of variables simultaneously. Effects of *T. versicolor* polysaccharides on treatment of diabetes in an animal (rat) model was investigated as well. Results have shown that the mechanism of action of these polysaccharides is similar to that of acarbose, inhibiting glucosidase enzymes.

2. Materials and Methods

2.1 Materials and chemicals

Potato dextrose agar (PDA), potato dextrose broth (PDB), KH_2PO_4, $MgSO_4$. $7H_2O$, $(NH_4)_2SO_4$, oleic acid, NaOH, HCl, ethanol absolute, H_2SO_4 98%, D-glucose, fructose, lactose and sucrose were purchased from Merck, Germany. Yeast extract and peptone were purchased from Ibresco, Iran. Soy milk and milk were provided by Kalleh, Iran. Acarbose, streptozotocin, p-NPG and α-glucosidase of *Saccharomyces cerevisiae* were purchased from Sigma-Aldrich, Germany.

2.2 Media and culture conditions

Iranian *T. versicolor* fungi isolated from forests in Mazandaran Province and the fungal mycelium were cultivated on PDA. Culturing time included five days at 28 °C. Then, cultures were transferred to seed culture media of PDB by punching out 10 mm^2 of the agar culture (seven days at 28 °C with agitation at 180 rpm) [20].

2.3 Selection of culture media for the growth of *Trametes versicolor* and production of EPS

Briefly, *T. versicolor* was cultured in a variety of culture media based on other studies [21,22]. Culture media were based on soy milk, cow milk and soy protein extraction. After autoclaving culture media, they were inoculated with 5% (v v^{-1}) of the seed culture (PDB) and incubated at 28 °C for seven days at 180 rpm using incubator (Jal Tajhiz, Iran). Initial pH of the culture media was adjusted to 6.15 by adding HCl (1 M) or NaOH (1 M). Studies have shown that the optimal pH for fungal growth and production of polysaccharides is 4-7 [23,24]. Table 1 shows compounds of the culture media, volume of the biomass and production of EPS. Then, growth of the fungi in culture media containing soy milk and four various carbon sources was investigated. Table 2 shows soy-based media with various carbon sources, volume of biomass and EPS production.

2.4 Optimization of fungal growth and polysaccharide production

To estimate effects of independent variables of glucose concentration, soy milk volume and initial pH on biomass and EPS production, response surface method by Box-Behnken experimental design was used (Design Expert software v.11). To calculate the optimal point for each variable, volumes of biomass (R1) and EPS (R2) were tested. Table 3 shows values of designed variables and volumes of biomass and EPS production.

2.5 Characterization of *Trametes versicolor* biomass and polysaccharides

Centrifuge (AWEL MF 20-R, France) was used to separate fungal biomass from the supernatant (5000 g, 15 min). Then, biomass was prepared for freeze-dry step (Operon, Korea). Supernatant and ethanol (96%) were mixed (1:4 v v^{-1}), incubated at 4 °C for 24 h and centrifuged at 11,000 g for 20 min. After removing the supernatant, ethanol (75%) was added to the tube and centrifuged at 11,000 g for 10 min and the percipitae was prepared for freeze-dry step [22]. After freeze-drying, weights of the biomass and EPS were measured.

2.6 *In vivo* rat models for anti-diabetes medicines

Streptozotocin is used to induce types 1 and 2 diabetes. For carrying out an animal study, 24 rats with 120 g of weight were randomly divided into four groups. Animals were housed in Animal House of the School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, under natural conditions of darkness, food and water. After a fasting night, animals were intraperitoneally injected (55 mg kg^{-1}) with freshly prepared streptozotocin solution in 0.01 M cold citrate buffer (pH 4.5) [25]. The healthy control group received a similar volume of citrate buffer. After induction of diabetes, fasting blood glucose (FBS) was assessed and glucose levels above 240 mg dl^{-1} were considered as measures to verify diabetic rats. The experiment was carried out 72 h after diabetic verification. The first group was non-diabetic (normal) group, the second group was diabetic that only received water, the third group received EPS of *T. versicolor* using daily gavage and the fourth group received a dose of 18 mg kg^{-1} of acarbose (daily pellet). After 21 days of treatment and 24 h after the last gavage, blood samples were collected from the hearts of the rats and transferred to Noor Pathological Laboratory, Tehran, Iran, for the assessment of FBS, triglycerides and cholesterol.

3. Results and Discussion

3.1 Selection of culture medium for the growth and production of EPS of *Trametes versicolor*

As described in Section 2.3, to find the optimal culture media and achieve the highest quantities of biomass and EPS in a short time, the *T. versicolor* was cultured in culture media with various sources of carbon, including glucose, fructose, lactose and sucrose and nitrogen sources, including peptone, soy isolate, cow milk and soy milk. Biomass and EPS production in PDB as a common culture medium for the fungi in various studies included 2.5 and 0.5 g l^{-1}, respectively. Lee et al. showed that the highest biomass and EPS production by *T. versicolor* occurred after ten days of culture [26]. In the present study, production of biomass and EPS in 10-day *T. versicolor* cultures was investigated. Results showed that in the media containing soybean extract, biomass and EPS productions reached 2.87 and 1.37 g l^{-1}, respectively. Tavares showed that YM (yeast malt extract) media was the best media of EPS production with 0.7 g l^{-1} and TaK media (Tien and Kirk media) was the best media of biomass production with 4.4 g l^{-1} [15]. Soybean extract, as a source of nitrogen, simultaneously increases *T. versicolor* biomass and EPS production in culture media. As seen in Table 1, use of this nitrogen source increased by almost 3 folds, compared to EPS production in PDB media.

In this study, *T. versicolor* was cultured on various carbon-source (glucose, fructose, lactose and sucrose) media with a concentration of 20 g l^{-1}. Experiments showed that quantity of biomass in synthetic media containing lactose included the highest value (3.53 g l^{-1}). The synthetic media, including fructose, sucrose and glucose, showed 2.81, 1.94 and 1.90 g l^{-1} biomass productions, respectively. The EPS produced in synthetic media, containing fructose, lactose, sucrose and glucose, increased to 6.87, 4.5, 2.5 and 1.23 g l^{-1}, respectively (Table 1). Addition of 0.13% v v^{-1} oleic acid increased EPS production by 7.74-fold; however, it did not affect biomass production. A study by Hao et al. on factors affecting growth of biomass and EPS production of *Schizophyllum commune* showed that addition of oleic acid to culture media increased EPS production by 58.33%. They concluded that oleic acid caused changes in the fungal cell wall composition and facilitated the entry of polysaccharides into culture media [27]. In the current study, addition of oleic acid increased EPS production. Based on the results, further studies on effects of fatty acids of the culture media on production volumes are suggested. In a study, *T. versicolor* New Zealand Wr74 was cultured using milk-based low-cost media in airlift bioreactors. Biomass, EPS and IPS productions included 8.9, 1.15 and 0.91 g l^{-1}, respectively [17]. Based on the present study, glucose and cow milk (50% v v^{-1}) were used to cultivate the fungi. Results showed that Iranian *T. versicolor* demonstrated significant growth in media containing milk and glucose. Biomass and EPS productions reached 18 and 4.25 g l^{-1}, respectively, which were significantly higher than those from New Zealand strain [17]. Cultivation of the fungi in soy milk (lactose-free milk) and glucose showed significant growth rates, compared to that in cow milk media and biomass and EPS productions increased to 24 and 4.2 g l^{-1}, respectively. Nitrogen source seems an important factor for the simultaneous enhancement of biomass and EPS productions.

Because *T. versicolor* in media containing soy milk included the highest biomass production, the media were selected as basic culture media in further steps. Lactose, fructose, sucrose and glucose were used to select the best carbon source for soy-based culture media. Results showed no significant differences between biomass and EPS productions (Table 2). In contrast to other studies on effects of carbon sources. A study by Arora et al. showed that use of sucrose as carbon source increased polysaccharide antioxidant activity. In general, disaccharides are more appropriate for EPS production because they facilitate polymerization [28,29]. In another study, Bolla et al. showed that various carbon sources (20 g l^{-1}) affected *T. versicolor* growth and EPS production. The highest quantity of biomass after seven days for media containing maltose was 7.6 g l^{-1}, followed by sucrose, glucose, fructose and lactose, respectively. The highest EPS production (8 g l^{-1}) was seen in media containing fructose, followed by the media containing maltose, sucrose, glucose and lactose, respectively. The lowest EPS production (2.8 g l^{-1}) was seen in media containing lactose [21]. Arteiro et al. reported that use of glucose and sucrose included no significant differences in production of *T. versicolor* polysaccharides [30]. Current results demonstrated that growth of Iranian *T. versicolor* significantly increased in soy milk and glucose media. Biomass and EPS productions included 20.5 and 6.5 g l^{-1}, respectively. Soy milk and glucose were selected because of their higher EPS production potentials.

3.2 Optimization of biomass and EPS productions

Based on the results from Section 3.1, soy milk culture media were selected as the best culture media to increase biomass and EPS productions. In culture media based on cow milk with adjusted pH, biomass production increased from 2.63 to 18.98 g l^{-1}, which showed a significant growth rate. The EPS production decreased from 4.97 to 4.9 g l^{-1} with no significance. Studies by Tavares et al. have shown that glucose can inhibit biomass and EPS productions by *T. versicolor* [15]. Moreover, other studies have shown that pH plays important roles in production of EPS by fungi [28]. Thus, it is necessary to further study effects of soy milk volume on biomass and EPS productions. To investigate effects of these three factors on biomass and EPS productions, the experimental design was carried out.

Table 1. Results of *Trametes versicolor* cultivation in synthetic immersion culture using various carbon sources

Test number	Compound of culture media	Amount of biomass (g l^{-1})	Amount of EPS (g l^{-1})
1	Peptone (1 g l^{-1}), Yeast Extract (2 g l^{-1}), K_2HPO_4 (1 g l^{-1}), $MgSO_4.7H_2O$ (0.2 g l^{-1}), $(NH_4)_2SO_4$ (2 g l^{-1}), Glucose (20 g l^{-1})	1.90	1.23
2	Peptone (1 g l^{-1}), Yeast Extract (2 g l^{-1}), K_2HPO_4 (1 g l^{-1}), $MgSO_4.7H_2O$ (0.2 g l^{-1}), $(NH_4)_2SO_4$ (2 g l^{-1}), Fructose (30 g l^{-1})	2.81	6.87
3	Peptone (1 g l^{-1}), Yeast Extract (2 g l^{-1}), K_2HPO_4 (1g l^{-1}), $MgSO_4.7H_2O$ (0.2g l^{-1}), $(NH_4)_2SO_4$ (2 g l^{-1}), Sucrose (20 g l^{-1})	1.94	2.5
4	Peptone (1 g l^{-1}), Yeast Extract (2 g l^{-1}), K_2HPO_4 (1 g l^{-1}), $MgSO_4.7H_2O$ (0.2 g l^{-1}), $(NH_4)_2SO_4$ (2 g l^{-1}), Lactose (20 g l^{-1})	3.53	4.5
5	Soybean extract (3 g l^{-1}), Yeast Extract (0.05 g l^{-1}), K_2HPO_4 (1 g l^{-1}), $MgSO_4.7H_2O$ (0.2 g l^{-1}), $(NH_4)_2SO_4$ (2 g l^{-1}), Glucose (20 g l^{-1})	2.87	1.37
6	Potato Dextrose Broth (24 g l^{-1})	2.5	6.5
7	Potato Dextrose Broth (24 g l^{-1}), Oleic acid (0.13% v v^{-1})	2.5	3.87
8	Soy milk (50% v v^{-1}), Glucose (20 g l^{-1})	24	4.2
9	Cow milk (50% v v^{-1}), Glucose (20 g l^{-1})	18	4.25

EPS= exopolysaccharide

Table 2. *Trametes versicolor* cultivation in soy-based media using various carbon sources

Compounds of culture media	Amount of biomass (g l^{-1})	Amount of EPS (g l^{-1})
Soy milk, Glucose	20.5	6.5
Soy milk, Fructose	20.63	5.4
Soy milk, Sucrose	20.81	5.9
Soy milk, Lactose	21.65	6.27

EPS=exopolysaccharide

In this study, concentration of glucose, pH and soy milk volume in *T. versicolor* culture media were assessed using response surface method (Table 3). The optimal point; in which, quantities of biomass (R1) and EPS (R2) simultaneously increased ($p<0.05$) was calculated using the software. Table 3 shows biomass and EPS produced in 17 experiments by combining various levels of the parameters.

Results showed that pH played an important role in simultaneous increasing of biomass and EPS productions. Studies have shown that differences in fungal morphology can affect mycelial growth and metabolite production [31-33]. In the present study, EPS production increased when there was a small pellet mycelium (star-like mycelium) in culture media. Acidic pH caused formation of dense pellets in *T. versicolor*, which affected EPS entry from the fungi to culture media. It is noteworthy that production of bioactive metabolites decreases at the extreme pH because the pH is associated with the permeability of the cell wall and membrane, causing adsorption of ions and removal of waste materials [28,29]. In a study by Que et al. with a two-step pH shift strategy in mid-growth from pH 6 to 4, EPS and laccase production increased to 8.2 g l^{-1} and 7680 U l^{-1}, respectively [16].

3.2.1 Optimization of biomass production by *Trametes versicolor*

The R^2 for biomass weight was 91.81%, which showed a good correlation coefficient. Based on the biomass weight, pH and volume of soy milk media with *p*-values of respectively 0.0232 and 0.0079 included significant effects on the quantity of biomass. Figure 1A shows effects of soya milk (ml) and pH on biomass production. Based on the analysis, no interactions were seen between the highlighted variables. The actual values were greater than the predicted ones of normal distribution. Effects of glucose (gl^{-1}) and soy milk (ml) on biomass production are shown in Fig. 1B. Also, Figure 1C shows effects of glucose (gl^{-1}) and pH on biomass production. Based on the analysis, no interactions were seen between the highlighted variables. The nonlinear regression model for biomass production based on the code variables was predicted based on the Eq. 1:

$$\text{Biomass} = 20.59 + 2.90A + 3.69B + 1.37C - 0.5825AB + 1.54AC - 2.54BC - 8.82A^2 + 2.76B^2 - 3.26C^2 \quad \text{(Eq. 1)}$$

3.2.2 Optimization of EPS production by *Trametes versicolor*

The R^2 for the production of EPS was 82%, with a correlation coefficient above 70% acceptable; therefore, this value was relatively appropriate. Based on the EPS production analysis, the initial pH with $p = 0.0004$ and the interaction of pH and soy milk with $p = 0.0129$ included significant effects on EPS production. Figure 2A shows effects of soy milk (ml) and pH on EPS production. In pH range of 4-4.5 and soy milk proportion of 65-75%, the highest EPS production was achieved. Effects of glucose (gl^{-1}) and soy milk (ml), and glucose (gl^{-1}) and pH on EPS production are shown in Figs. 2B and 2C, respectively. In this study, fungi with a small pellet morphology and sprawling hyphae in form of the sun increased the quantity of EPS in culture media. Analysis showed that the actual and the predicted values for EPS production were close. The nonlinear regression model for EPS production based on the code variables was predicted based on the Eq. 2

$$\text{EPS} = 7.01 - 3.34A - 0.7731B - 0.6348C - 2.70AB + 1.91AC + 1.47BC \quad \text{(Eq. 2)}$$

Table 3. Designation of Box-Behnken and its results based on the biomass weights and exopolysaccharide contents

Test number	Parameters			Results	
	A: pH	B: Soy milk volume (ml)	C: Glucose concentration (g l^{-1})	R1: Amount of biomass (g l^{-1})	R2: Amount of EPS (g l^{-1})
1	4	25	20	10.53	7.66
2	8	25	20	13.79	6.66
3	4	75	20	16.46	12.33
4	8	75	20	17.39	0.533
5	4	50	5	2.86	15.06
6	8	50	5	9.3	4.27
7	4	50	35	4.66	6.2
8	8	50	35	17.26	3.04
9	6	25	5	12.26	8.9
10	6	75	5	27.35	3.6
11	6	25	35	17.93	8.47
12	6	75	35	22.86	9.04
13	6	50	20	20.77	4.75
14	6	50	20	21.04	7.4
15	6	50	20	19.32	8.2
16	6	50	20	22.3	7
17	6	50	20	19.54	4.8

EPS=exopolysaccharide

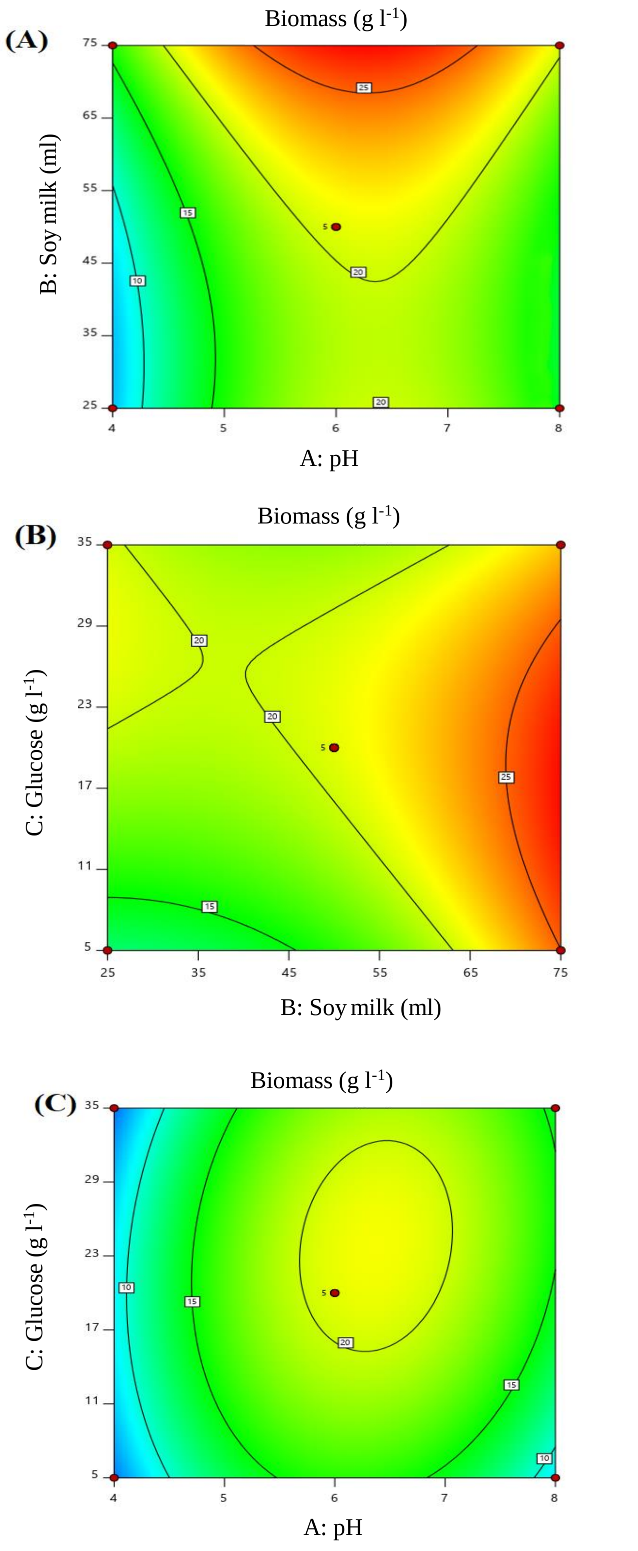

Figure1. Effect of (A) pH (p = 0.232) & soy milk volume (p = 0.0079), (B) soy milk &glucose, (C) pH & glucose on biomass weight

3.2.3 Finding optimal points and checking dry cells and EPS contents

Design-Expert software was used to optimize production of biomass and EPS. At pH 4.66 with 13 g l^{-1} glucose and 75% v v^{-1} soy milk, biomass and EPS respectively included 21.90 and 10.40 g l^{-1}, with predicted confidence of 73% (Fig. 3). To verify this point, an experiment was carried out under optimum conditions and results showed that biomass and EPS productions included 21.80 and 9.6 g l^{-1}, respectively. Because of no clear relationships between the biomass and EPS productions, it is necessary to identify important factors contributing to increased coproduction of biomass and EPS in *T. versicolor*.

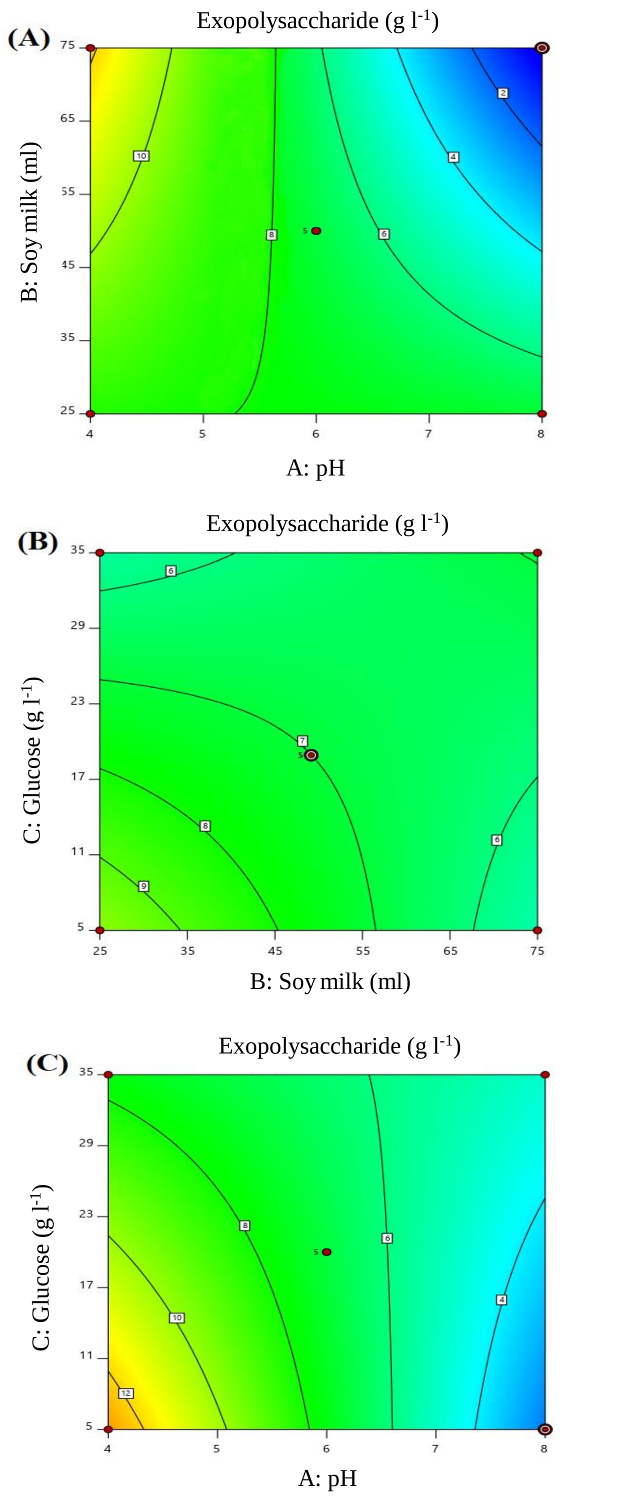

Figure 2. (A) effects of pH and volume of soy milk (p = 0.0129), (B) effects of soy milk and glucose and (C) effects of pH (p = 0.0004) and glucose on the quantity of exopolysaccharides

3.4 *In vivo* test of *Trametes versicolor* exopolysaccharides

As described in Section 2.6, triglycerides, cholesterol, LDL and HDL were assessed. In diabetic control group, significant increases were seen in FBS, triglycerides, cholesterol, LDL and HDL, compared to the healthy group (Table 4). Diabetic treatment with streptozotocin increased these factors as well as FBS. Analysis of data from the rat blood tests was carried out using Excel 10 software and the mean and standard deviation were calculated. To compare results, t-test and Excel 10 software were used. The test showed significant differences between the results of FBS in treated diabetic rats and EPS and acarbose, compared to those in rats of the control group with a *p*-value of less than 0.05. Results demonstrated that daily intake of 400 mg kg^{-1} of EPS from *T. versicolor* in diabetic group decreased FBS by 50.8%, compared to FBS in diabetic group. This also decreased triglycerides by 89% and cholesterol by approximately 20%, increased HDL by 5.12% and decreased LDL by 21.67%. In standard group treated with acarbose, glucose decreased by 13.77%, triglycerides by 69% and cholesterol by 9.5%. Furthermore, HDL increased by 2.5% and LDL decreased by 28% in diabetics.

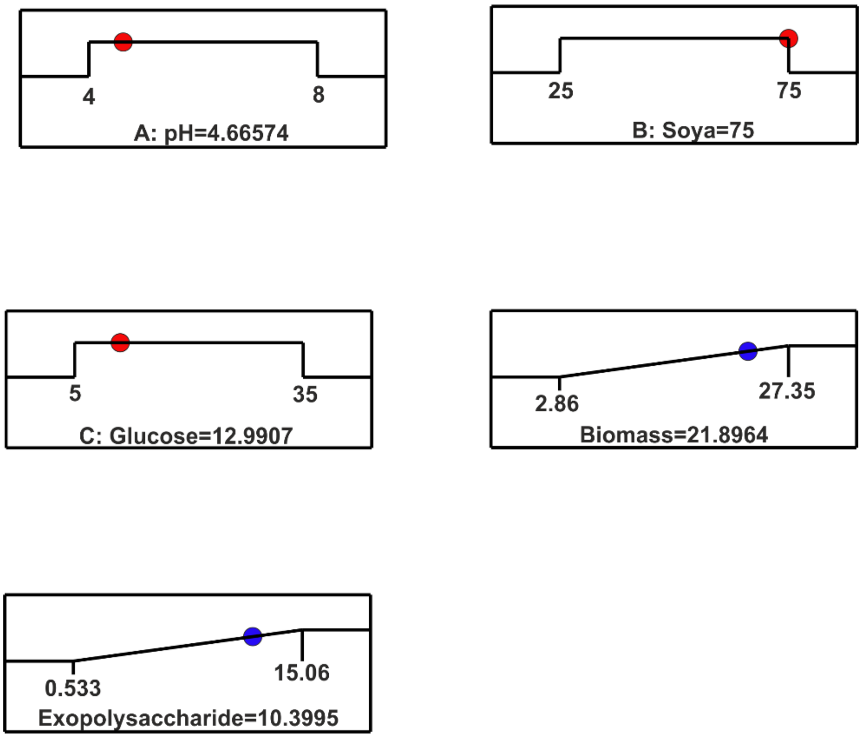

Figure 3. Assessment of the optimal spots to increase fungal growth and production of exopolysaccharides

Table 4. Average results and standard deviation obtained from blood tests in the animals

Groups	Glucose (mg dl^{-1})	Triglyceride (mg dl^{-1})	Cholesterol (mg dl^{-1})	HDL (mg dl^{-1})	LDL (mg dl^{-1})
Normal	138 ±1.41	56.5 ±2.12	55.5 ±0.70	30.5 ±0.70	5 ±2.12
Diabetic	653 ±1	560.5 ±07	79.66 ±1.15	39 ±1	7.66 ±1.52
Diabetic+ EPS	324.66 ±1.52*	62.5 ±2.1*	63 ±23*	40.66 ±1.52	8 ±2
Diabetic+acarbose	565 ±2.82	173 ±1.41	71.5 ±0.70	31 ±1.41	5.5 ±0.7

*$p < 0.05$ versus diabetic group. EPS= exopolysaccharide

Results showed that Iranian *T. versicolor* included higher biomass and EPS productions, compared to that the Chinese, New Zealand and commercial strains did. Further studies on EPS glycemic control in streptozotocin-induced diabetic rats showed that a daily dose of 400 mg kg^{-1} decreased blood glucose concentration by 38.8%, compared to controls. Results of the common drug use of acarbose (18 mg kg^{-1}) demonstrated that acarbose decreased FBS by 13.47%. In 2017, experiments by Shokrzadeh et al. showed that IPS extracted from *T. versicolor* fruiting body (500 mg kg^{-1}) decreased the glucose level of streptozotocin-induced diabetic rats by approximately 14% after three weeks [34]. Zhang et al. found that a 200 mg kg^{-1} dose of IPS from *T. versicolor* cultured in 0.75% of soy milk and 3% of glucose decreased glucose level (30.7%) in streptozotocin-induced diabetic rats after three weeks [35]. In 2008, Yang et al.

reported that EPS of *T. versicolor* cultured in PDB media at 100 mg kg^{-1} doses for two weeks decreased glucose levels by 29.9% in streptozotocin-induced diabetic rats [36]. A comparison of the results has shown that use of mushrooms grown in submerge cultures includes significant effects on blood glucose levels, compared to the form of fruiting body.

Studies by Wang et al., showed that increases in D-glucose levels in glucose-lowering polysaccharide compounds increased efficiencies of these compounds and blood glucose levels decreases dramatically [8]. The researchers showed that increases in glucose levels of these compounds increased binding of these compounds to α-glucosidase enzyme in the intestinal membrane. Changes in culture media or uses of the mycelium or fruiting body change these compounds and hence affect blood glucose levels [9]. Hsu et al. purified polysaccharides from *T. versicolor* using various chromatographic techniques and increased inhibition of α-glucosidase enzyme for the treatment of diabetes [14]. One of the most important complications of diabetes is a significant increase in triglyceride and cholesterol levels due to an impaired metabolism. Therefore, finding novel drugs with higher efficiencies in lowering lipids is one of the big pharmacists' concerns. Acarbose decreased triglycerides by 69% and cholesterol by 9.49%. The EPS decreased triglycerides by 89% and cholesterol by nearly 20%. Results showed that EPS appropriately decreased blood lipids. Moreover, results showed that HDL (or good cholesterol) increased by 5.12% in EPS-treated group, compared to control group. Treatment of rats with acarbose increased HDL by 2.5%. In fact, LDL (or bad cholesterol) decreased by 21.67% in groups treated with EPS, compared to control group. Indeed, LDL decreased by 28% in groups treated with acarbose. Therefore, results indicate that use of polysaccharide significantly decreases blood glucose, triglyceride and cholesterol levels while increases HDL levels.

4. Conclusion

Recently, *T. versicolor* polysaccharide compounds have been used in diabetes research. Up to now, diabetes testing has been limited to the clinical phase of testing animal models. In this study, Iranian *T. versicolor* was cultivated in various submerge cultures. Results showed that culture media containing soy milk were appropriate media for biomass and EPS productions. Optimization of the culture media was carried out using response surface method, 13.01 g l^{-1} glucose, 75% v v^{-1} soy milk and pH 4.67. In the media, biomass and EPS production included 21.7 and 9.6 g l^{-1}, respectively, while biomass and EPS productions in PDB media included 2.5 and 0.5 g l^{-1}, respectively. Cultivation of *T. versicolor* in these culture media increased biomass production by 8.7 and EPS production by 19.2 folds, compared to mushroom cultivation in PDB media. In the current study, a high quantity of 20 g l^{-1} of *T. versicolor* biomass was achieved for the first time. In general, results showed that the best media for the growth of *T. versicolor* was soy-based media. Results also demonstrated that pH, as an important factor in addition to the volume of soy milk, included important roles in increasing EPS production. Results from diabetic animal model showed that EPS treated rats included significantly decreased glucose levels (50.38%) and their blood lipid levels were improved within 21 days. In conclusion, EPS of Iranian *T. versicolor* could be used as a good drug supplement for the treatment of diabetes.

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6. Conflict of Interest

The authors declare no conflict of interest.

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بهینه‌سازی محیط کشت برپایه سویا به‌منظور تولید پلی‌ساکاریدهای برون‌سلولی با فعالیت زیستی توسط قارچ دارویی *ترامیتیس ورسیکالر*

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چکیده

سابقه و هدف: دیابت ملیتوس پنجمین عامل مرگ و میر در جهان به شمار می‌رود. عوارض پیشرفته متابولیکی و ضایعات اندام‌های گوناگون از اثرات زیان‌آور دیابت می‌باشد. بیشتر از ٪۹۰ موارد دیابت به دیابت نوع ۲ تعلق دارد. چندین دهه است که قارچ‌های دارویی مانند *ترامیتیس ورسیکالر* به عنوان درمان دیابت مطرح شده‌اند. پلی‌ساکاریدهای برون سلولی قارچی در غشای روده‌ای فعالیت آلفا گلوکوزیدازی از خود نشان می‌دهند. تحقیق حاضر به بهینه‌سازی تولید پلی‌ساکاریدهای این قارچ و بررسی اثر آن بر مدل حیوانی می‌پردازد.

مواد و روش ها: در مطالعه حاضر، *ترامیتیس ورسیکالر* ایرانی در محیط‌های کشت گوناگون بر پایه شیر سویا، شیر گاو و عصاره پروتئینی سویا کشت داده شد. پس از انتخاب محیط کشت مناسب برای تولید زیست‌توده[۱] و پلی‌ساکارید برون‌سلولی[۲]، روش سطح پاسخ با طراحی باکس-بنکن برای بررسی اثرات متغیرهای مستقل گلوکز ($g\ l^{-1}$)، شیر سویا ($v\ v^{-1}$) و pH بر تولید زیست‌توده و پلی‌ساکارید برون سلولی و بهینه‌سازی آن‌ها استفاده شد. مدل حیوانی (موش صحرایی) مبتلا شده به دیابت در اثر استرپتوزوتوسین برای بررسی پلی‌ساکاریدهای برون سلولی بر دیابت مورد استفاده قرار گرفت. موش‌ها به مدت ۲۱ روز با پلی‌ساکاریدهای برون سلولی مورد تیمار قرار گرفتند.

یافته‌ها و نتیجه‌گیری نتایج نشان داد که محیط‌های کشت حاوی شیر سویا برای رشد و تولید فرآورده‌های قارچی مناسب بودند. تولید زیست‌توده و پلی‌ساکاریدهای برون سلولی به ترتیب ۲/۸۷ و ۱/۳۷ گرم بر لیتر افزایش یافت. بر اساس تجزیه و تحلیل روش سطح پاسخ، *p* برای pH برابر ۰/۰۰۰۴ و برای برهمکنش pH و سویا ۰/۰۱۲۹ بود که بر اثرات معنی‌دار آن‌ها بر تولید پلی‌ساکاریدهای برون سلولی اشاره دارد. در شرایط بهینه محیط‌های کشت با pH ۴/۶۷، میزان گلوکز ۱۳/۰۱ گرم بر لیتر و شیر سویا ۷۵ درصد حجمی حجمی، تولید زیست‌توده و پلی‌ساکارید برون سلولی به ترتیب به ۲۱/۸۰ و ۹/۶ گرم بر لیتر رسید. در مدل موش مبتلا شده به دیابت با استفاده از استرپتوزوتوسین، کاهش ۵۰/۳۸ درصدی در گلوکز خون مشاهده شد، در حالی‌که برای تری گلیسریدها، کلسترل و لیپوپروتئین‌های با چگالی کم این کاهش به ترتیب ۸۹، ۲۰ و ۲۱/۶۷ درصد بود. همچنین، لیپوپروتئین‌های با چگالی زیاد تا حدود ۵/۱۲ درصد افزایش یافت.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

1 Biomass
2 Exopolysaccharide