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Kinetic Modeling of Growth and Mycelial Exopolysaccharide Production by *Lentinus edodes* (Shiitake Edible Mushroom)

Ashrafalsadat Hatamian-Zarmi [1*], Zahra Tasharofi[1], Hale Alvandi[1], Mohammad Barshan-tashnizi[1], Bahman Ebrahimi-Hosseinzadeh[1], Zahra Beagom Mokhtari-Hosseini[2]

1- Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran.
2- Department of Chemical Engineering, Faculty of Petroleum and Petrochemical Engineering, Hakim Sabzevari University, Sabzevar, Iran.

Abstract

Background and Objective: *Lentinus edodes* (Shiitake) is a rich source of secondary metabolites, including exopolysaccharides. These compounds strengthen the immune system and play essential roles in prevention and treatment of several diseases, including cancers. A way to increase production of polysaccharides is the use of elicitors. Examples of these elicitors include microbial volatile organic compounds, which are produced in microo-rganism co-cultures. The objective of this study was to investigate effects of these compounds on production of Shiitake exopolysaccharides.

Material and Methods: To decrease cultivation time, Shiitake was cultured in four culture media, including (1) potato dextrose broth, (2) potato dextrose broth and D-glucose, (3) malt extract broth and (4) malt extract broth and D-glucose. After selecting appropriate culture media, fungal growth curve, kinetic growth of pellets and filamentous morphology were studied. Novel method of simultaneous aerial co-culture was used to increase production of Shiitake exopolysaccharides, which acted as an elicitor by inducing microbial volatile organic compounds of other microorganisms. Microbial volatile organic compounds were analyzed using gas chromatography-mass spectroscopy.

Results and Conclusion: Malt extract medium containing glucose was selected for submerged and solid cultures of Shiitake and the growth time decreased to 18 d. Shiitake biomass production included 11 $g.l^{-1}$. Filamentous morphology included higher production rates due to higher surface-to-volume ratios, compared to that the pellet morphology did. Shiitake fungal biomass and exopolysaccharides in co-cultures with *Aspergillus niger* included 14 and 4 $g.l^{-1}$, respectively. Furthermore, biomass and exopolysaccharides included 11 and 4.7 $g.l^{-1}$ in co-cultures with *Schizophyllum commune*, respectively. Microbial volatile organic compounds produced by *Aspergillus niger* and *Schizophyllum commune* in co-cultures, as elicitors, increased biomass and exopolysaccharide productions in Shiitake. Therefore, it suggests that microorganism co-cultivation is a low-cost effective method for Shiitake exopolysaccharide production.

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***Corresponding author:**

Ashrafalsadat Hatamian-Zarmi*
Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran.

Tel: +98-21-61118503

E-mail:

hatamian_a@ut.ac.ir

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1. Introduction

Lentinus edodes (Shiitake), as a cap basidiomycete, is one of the most common fungi that has been cultivated for thousands of years. Shiitake mushroom includes desirable tastes and bioactive compounds with healing characteristics. This edible-medicinal mushroom is currently the most cultivated mashroom globally after *Agaricus bisporus* (white button mushroom) and its production reaches more than 2 million tons per year. Shiitake is a rich source of various carbohydrates, proteins, vitamins and minerals [1]. Secondary metabolites of this fungus include polysaccharides. Shiitake polysaccharides include glucose, mannose, xylose, galactose, fucose, rhamnose and arabinose [2]. Polysaccharides of this fungus demonstrate various therapeutic characteristics such as antioxidant, antibacterial, anti-cancer

and anti-diabetic characteristics and strengthen the immune system [3,4]. Observations show that variables such as culture media composition, temperature and pH affect mycelial growth of the fungi. Effects of these factors on the production of secondary metabolites and characteristics of the fungal metabolites can be studied and optimized [5-9]. A group of the compounds that affect growth and production of the polysaccharides include elicitors. Elicitors include biological or non-biological origins that induce biosynthesis and storage of secondary metabolites by inducing host defense responses. Non-bioelicitors include ultraviolet light, ultrasound and chemical compounds such as dimethyl sulfoxide [10-12]. Bioelicitors include cellular components of fungi, plants and microorganisms, which may include specific compounds such as chitin or a mixtures of biological compounds such as mushroom extractions. Microbial volatile organic compounds (MVOCs) consisting of low molecular weight molecules can be used as elicitors. These compounds are resulted from the primary and secondary metabolism of microorganisms. Effects of these compounds can be investigated by simultaneous culture of the microorganisms [13,14].

Up-to-date, several studies have been carried out on fungal metabolite production in co-cultures [15-17]. Simultaneous aerial co-culture refers to cultivation of physically separated microorganisms, which are only connected by air and their vapor organic compounds affect each other. A similar process is seen in nature and the compounds of microorganisms cause defense responses and stimulate production of secondary metabolites [18,19]. Simultaneous aerial co-culture between four various soil bacteria and *Pseudomonas fluorescens* stimulates production of antimicrobial secondary metabolites in these bacteria [20]. Venkatarman et al. showed that the MVOC (3,2-butanediol) increased production of secondary metabolites (pyocyanin and pyoverdine) in *P. aeruginosa* [21]. Savoie et al. reported that addition of *Trichoderma* spp. to culture media of Shiitake mushrooms increased activity of the laccase enzymes by 3-30 times [22]. Co-cultivation with activation of internal signals results in activation of secondary metabolite genes [23]. Production of *Ganoderma lucidum* polysaccharides in aerial co-cultures with *Pleurotus oestrus* increases by 2.2 times after 6 d and reaches 3.35 $g.l^{-1}$ under optimal conditions [19]. Kalantari et al. investigated that aerial co-cultures of *G. lucidum* with *Bacillus subtilis* and *P. aeruginosa* decreased growth of mycelia of this fungus. Production of ganoderic acid in this fungus increased more than 2.8 times [18]. Therefore, the aim of this study was to investigate polysaccharide production of Shiitake using elicitors. First, the optimal culture medium for the Shiitake growth was selected. Then, effects of MVOCs from various microorganisms on the growth rate of Shiitake biomass and exopolysaccharide (EPS) production were investigated.

2. Materials and Methods

2.1. Preparation of microorganisms

Microorganisms were provided by China General Microbial Culture Collection Center (CGMCC), University of Tehran Microbial Technology and Research Center (UTMC) and Persian Type Collection Center (PTCC) in solid media. *Lentinus edodes* was provided by the Microbial Bank of Tarbiat Modares University, Tehran, Iran (Table 1). This study used nutrient agar media (Merck, Germany) for the solid cultures of bacteria and potato dextrose agar media (Merck, Germany) for fungi. For submerged cultures, bacteria were cultured in nutrient broth media (Merck, Germany) and fungi were cultured in potato dextrose broth (PDB) media (Merck, Germany). For the cultivation of each microorganism, inoculation quantity was 10% v v^{-1} and culture media were incubated at 28 °C and 150 rpm.

2.2. Selecting appropriate culture media for Shiitake mushrooms

The first aim of this study was investigation of Shiitake growth and EPS production in various culture media. Potato dextrose agar was used for solid cultivation of Shiitake. After the cultivation of Shiitake in solid media, a square of the culture (1 × 1 cm^2) was cut off and added to PDB culture media. The PDB was used for submerge cultivation of Shiitake (seed culture). Shiitake EPS production was studied in four culture media. These culture media included PDB, PDB containing 17 $g.l^{-1}$ of D-glucose, malt extract broth (MEB) and MEB containing 17 $g.l^{-1}$ of D-glucose. In this study, 17 $g.l^{-1}$ of D-glucose were used as the elicitor for polysaccharide production. Quantity of inoculation for each culture medium was 10% v v^{-1} from seed cultures. Culture media were incubated at 28 °C for 30 d at 150 rpm [13,24].

2.3. Growth curve of Shiitake mushrooms

To plot the growth curve, Shiitake was cultured in 100-ml flasks containing 20 ml of the selected medium. After inoculation (200 µl of Shiitake seed culture in PDB), flasks were transferred into a shaker incubator and incubated at 28 °C for 20 d at 150 rpm (Jaltjahiz, Iran) .

In each sample, fungal biomass was isolated using centrifuge (5000× g for 20 min). After lyophilized the biomass using freeze-dryer (Operon, South Korea), weight of each sample was measured using scale. Based on a previous study by the authors, ethanol (4:1 v:v) was mixed with the supernatant to extract the fungal EPS. After storing at 4 °C for 24 h, EPS was separated using centrifuge (5000× g for 10 min) and lyophilized [5].

Table 1. Microorganisms used for co-cultures in this study

Fungi	Bacteria
Auricularia auricula-judae (Jewish ear)	*Bacillus subtilis* (PTCC 1247)
Aspergillus niger (UTMC 5018)	*Escherichia coli* (PTCC 1269)
Agrocybe (gift)	*Staphylococcus aureus* (UTMC 1429)
Candida albicans (UTMC 5024)	
Chinese *Ganoderma lucidum* (CCGMC 5.616)	
Fomes fomentarius (NCBI MG835861)	
Fusarium solani (PTCC 5284)	
Fusarium sporotrichioides (IBRC-M 30197)	
Hericium beech (NCBI MW136052)	
Hericium chestnut (gift)	
Iranian *Ganoderma lucidum* (IBRC-M 30306)	
Lentinus edodus (Tarbiat Modares University 25. Shibel)	
Mucor Himalayas (PTCC55)	
Neurospora intermedia (PTCC 5291)	
Pleurotus ostreatus (FNST-203)	
Schizophyllum commune (NCBI MG761830)	
Trametes gibbosa (gift)	

PTCC: Persian Type Culture Collection (http://ptcc.irost.org/).
IRBMC: Iranian Biological Resources Center (http://www.ibrc.ir).
CCGMC (CGMCC): Center for Collection of General Microbiological Cultures.
FNST: Faculty of New Sciences and Technologies

2.4. Kinetic model of Shiitake growth

Multiple flasks were inoculated with 10 ml of the pellet mycelia and incubated. Three flasks were randomly selected for sampling each day. Spherical mycelial cultures normally appeared to follow the kinetics of cubic roots. Emerson biosynthetic expression was used for the microbial growth calculations in Eq 1:

$$M^{1/3} = k_c t + M_0^{1/3} \quad \text{Eq (1)}$$

Where, M was the quantity of total mass (g), k_c was the constant (g $^{1/3}$ × h^{-1}) and t was the time. [25]. Logistic equation was used to adapt to the biomass curve. This equation was independent of the culture media substrate and widely used as an unstructured model to explain the cell growth as Eq 2:

$$\frac{dC}{dt} = \mu_{max}\left(1 - \frac{C}{C_m}\right) C \quad \text{Eq (2)}$$

Where, C was the biomass (g.l^{-1}), μ_{max} was the maximum specific growth rate (h^{-1}) and C_m was the maximum quantity of biomass that could be achieved (g.l^{-1}) [25]. Luedeking-Piret model was used for the EPS curve. This equation was widely used to predict metabolites as follows:

$$\frac{dP_x}{dt} = \alpha \frac{dC_x}{dt} + \beta C_x \quad \text{Eq (3)}$$

Where, P_x was the EPS concentration (g.l^{-1}), C_x was the cell mass (g.l^{-1}) and α (g × g $^{-1}$) and β (g × (g × h) $^{-1}$) were the constant values. This was an experimental model; in which, dC_x/dt showed production of EPS relative to the growth rate and C_x represented production of EPS regardless of growth [25,26].

2.5. Aerial co-culture of Shiitake mushrooms with microorganisms

Shiitake mushrooms were cultured alone in a flask and 21 microorganisms, including Shiitake fungi alone, three species of bacteria and 17 species of other fungi, were separately cultured in other flasks. Each two flasks were connected to each other using glass tubes inserted in the lids. Indeed, Flask 1 included Shiitake mushrooms and Flask 2 included Shiitake mushrooms or other 20 microorganisms. Therefore, production of MVOCs in Flask 2 affected polysaccharide production in Flask 1 (Figure 1).

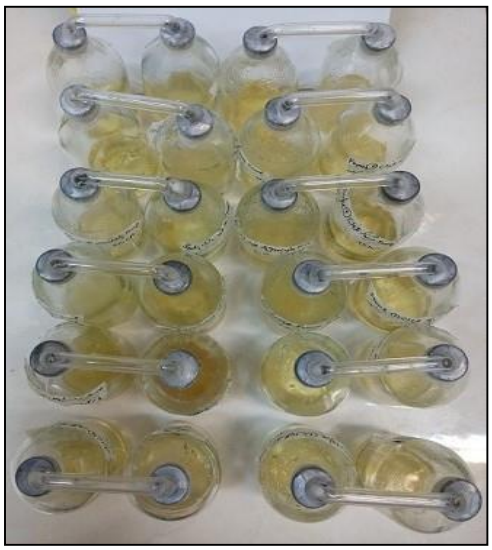

Figure 1. Flasks of Shiitake aerial co-cultures with other microorganisms

Inoculation quantity was 10% v v^{-1} and culture media were incubated at 28 °C for 20 d at 150 rpm. Then, 50 ml of MEB media with glucose for Shiitake mashrooms, 50 ml of nutrient broth for bacteria and 50 ml of PDB for other fungi were added to the flasks. As previously described, biomass and EPS were isolated [18,19].

2.6. Gas chromatography-mass spectrometry analysis for microbial volatile organic compounds

Gas chromatography-mass spectrometry analysis (GC-Mass analysis) was carried out to assess MVOCs produced and used in aerial co-cultures by Shiitake mushrooms and *Schizophyllum* (*S.*) *commune* or *Aspergillus* (*A.*) *niger*. This analysis was carried out for the microorganisms that increased growth and production of Shiitake EPS to identify MVOCs with positive effects on the growth and production of EPS. Therefore, Shiitake mushrooms were co-cultured with *S. commune* or *A. niger*. Submerged cultures of Shiitake mushrooms were used to assess Shiitake volatiles. Twenty days later, all samples were transferred to the laboratory, Department of Chemistry, Chemical Research Institute of Shahid Beheshti University, Tehran, Iran, for GC-Mass analysis [27]. To carry out GC-Mass analysis, Shiitake mushrooms were co-cultured with *S. commune* and *A. niger*. After a 20-d culture period, samples were transferred into a 70 °C oven for several hours and prepared for the analysis. The SPME fiber adsorbents were used. Detentions of capillary 9 column included 30 m, 0.2 mm and 0.33 µm. The carrier gas was helium with a flow rate of 1 $ml.min^{-1}$. The mass range was 35-500 a.m.u. Ionization was carried out by electron impact at 70 eV with an ion source temperature of 200 °C [27]. The GC-Mass analysis was carried out using Agilent 7890A Instrument (Agilent Technologies, USA) with Model 5977A Mass Spectrometer Detection System. The injection temperature was 25 °C and the volume of injection was 1 µl. Temperature was set at 50 °C for 1 min, then heated to 220 °C at 3 °C/min and held for 3 min [28].

2.7. Statistical analysis

Each experiment was carried out with three replications. Kinetic modeling was carried out using Minitab Software v.18. In general, $p < 0.05$ were reported as statistically significant ($0.01 < p < 0.05$).

3. Results and Discussion

3.1. Appropriate culture media for Shiitake mushrooms

Cultivation time of Shiitake mushrooms in PDA and PDB were 30 and 28 d, respectively. This was a relatively long time for mushrooms to grow. Therefore, D-glucose was used as an elicitor in culture media; however, the biomass growth rate did not change significantly. In the next step, MEB and MEA media were used to cultivate Shiitake mushrooms and D-glucose was added to the culture media. Various concentrations of sugar were used in various culture media. These concentrations could range 5-50 $g.l^{-1}$ [6,25,29]. Biomass growth in PDB, PDB and D-Glucose and MEB included 2.5, 4 and 7 $g.l^{-1}$, respectively. Results showed that the malt extract media containing D-glucose included the highest biomass production within the shortest time. Biomass growth rate in MEB media containing D-glucose (MEB.G) reached 11 $g.l^{-1}$ after 18 d ($p < 0.05$). Studies have been carried out to improve physicochemical parameters of Shiitake growth. Elisashvili et al. (2004) studied effects of various carbon and nitrogen sources on cultivation of Shiitake mushrooms. Biomass production of this fungus reached its maximum when gluconate or glucose was used as a unique carbon source [30]. In a study, Feng et al. (2010) cultivated Shiitake mushrooms in synthetic media at pH 5.5–5 and 28 °C. Biomass production of this fungus was doubled after 20 d and reached 6.88 $g.l^{-1}$ [25]. Use of MEB and D-glucose media [25,29,30] was more efficient and economically viable for EPS production in Shiitake mushrooms, compared to other studies.

3.2. Shiitake growth curve

Figure 2 shows the growth curve of Shiitake mushrooms in 20 d. Shiitake biomass growth began from Day 3. Up to 10-12 d, growth phase was followed by a steep slope; after which, relative decreases in growth rate were seen. Growth continued until Day 18 and reached 11 $g.l^{-1}$. Biomass was measured until Day 20, showing that the growth rate did not change (stationary phase). From Day 10 of the growth, production of secondary metabolites, including EPS, began and continued until Day 20, reaching its highest level (4.51 $g.l^{-1}$). No clear relationships were detected between the mycelial growth and EPS production. The relationships could be positive or negative in various fungal species. In the present study, linear relationships were reported between the biomass and EPS production [6,25,31].

3.3. Investigation of kinetic parameters of Shiitake mycelial growth

Naturally, fungi show various morphologies in submerged culture media, majorly in filamentous and pellet masses. Morphology affects mycelial growth and production of metabolites. In the study on pellet morphology, root-cube kinetic equation was used to simulate changes in biomass and loading-pyrite equation was used to simulate the EPS concentration as Eq 4.:

$$C(t) = (kt + C_0^{1/3})^3 \qquad \text{Eq (4)}$$

This equation could be written as an alternative to Eq. 3 to obtain Eq.5:

$$P(t) = P_0 + \frac{1}{4}\beta k^3 t^4 + \left(\alpha k^3 + C_0^{1/3}\beta k^2\right)t^3 + \left(\frac{2}{3}C_0^{2/3}\beta k + 3C_0^{1/3}\alpha k^2\right)t^2 + (C_0\beta + 3C_0^{2/3}\alpha k) \qquad \text{Eq (5)}$$

Regression values in the pellet morphology were negative, indicating that the pellet morphology was not responsive in this experiment.

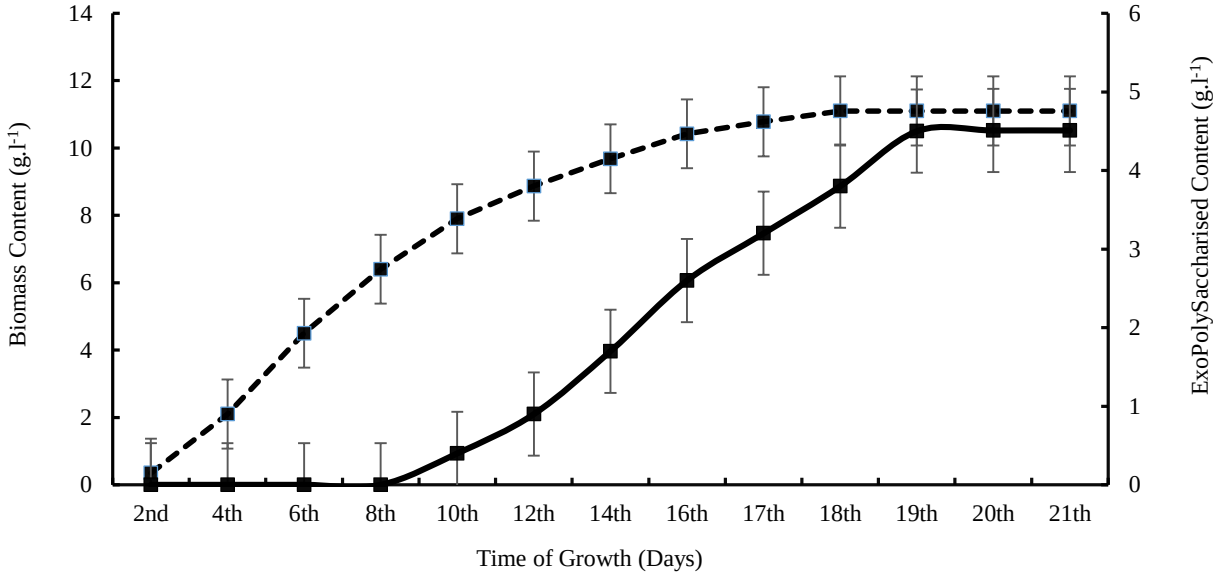

Figure 2. Growth curve of the Shitake mushroom within 20 days

As seen in Figure 3a, the growth curve of Shiitake mushrooms was a kind of S-shaped curves (sigmoidal curves) and the exponential phase began after 2 d. The EPS production began after 10 d.Biomass production increased until Day 14 and the maximum EPS production occurred on Days 14-16. Figure 3b shows Shiitake EPS production within 20 d. In the study on filamentous morphology, logical equation independent of the substrate was used to simulate changes in biomass growth and loading-pyrite equation was used to simulate EPS production. Equation (6) was as:

$$C(t) = \frac{C_m}{1+((C_m/C_0)-1)e^{-\mu m t}} \quad \text{Eq (6)}$$

This equation could be written as an alternative to Eq (3) :

$$P(t) = P_0 + \alpha\,[C(t) - C_0] + \beta C_m\,\{\ln[C_0/C(t)] + \mu_m t\}\,/\mu_m \quad \text{Eq (7)}$$

Literatures show that a relatively little research has been carried out on effects of fungi morphologies on metabolite production. Fungal growth was observed in pellet morphology with increasing sizes and in filamentous morphology with increasing hyphal lengths. Therefore, root-cube growth might be associated to pellet shapes and exponential growth to filamentous morphology. Results showed that the filamentous morphology was appropriate for the scale-up. Although the nonstructural model was mathematically and theoretically further plausible and easily validated, the structural model seemed more comprehensive in the fundamental aspects of structure, function and composition [6,25,31]. Table 2 demonstrates the regression values. Kinetic characteristics of the biomass growth and EPS production were well explained using this model (R^2 = 0.9847 and R^2 = 0.9941, respectively). Constant quantity of the alpha dependent on growth was more significant than the constant quantity of non-growth dependent beta, showing linear relationships between the biomass and EPS. Thus, production of EPS depended on growth. Negative coefficients of β showed that growth-dependent production increased in contrast to non-growth-dependent production when the total quantity of EPS was constant. In general, results showed that filamentous morphology included higher surface-to-volume ratios than that the pellet morphology did as well as higher production rates within shorter times with a specific growth rate of maximum biomass production (μ_m) [25]. The kinetic model can control a system for Shiitake fermentation, including determining duration of the mycelial production period and predicting the process. It is noteworthy that the kinetic coefficients vary based on the processes of particular fermentations.

3.4. Simultaneous aerial co-cultivation

Figure 1 shows Shiitake aerial co-culture flasks with other microorganisms. Almost all the microorganisms used in simultaneous aerial co-culture with Shiitake mushrooms included significant effects on the mushroom growth. Effects of the simultaneous aerial co-culture of fungi on growth of Shiitake mushrooms were further significant. Figure 4 shows biomass production in aerial co-cultivation of Shiitake mushrooms alone and with various microo-rganisms. The biomass growth rate increased from 11 $g.l^{-1}$ in PDB to 14.5 $g.l^{-1}$ in simultaneous aerial co-culture with *S. commune* ($p < 0.05$).

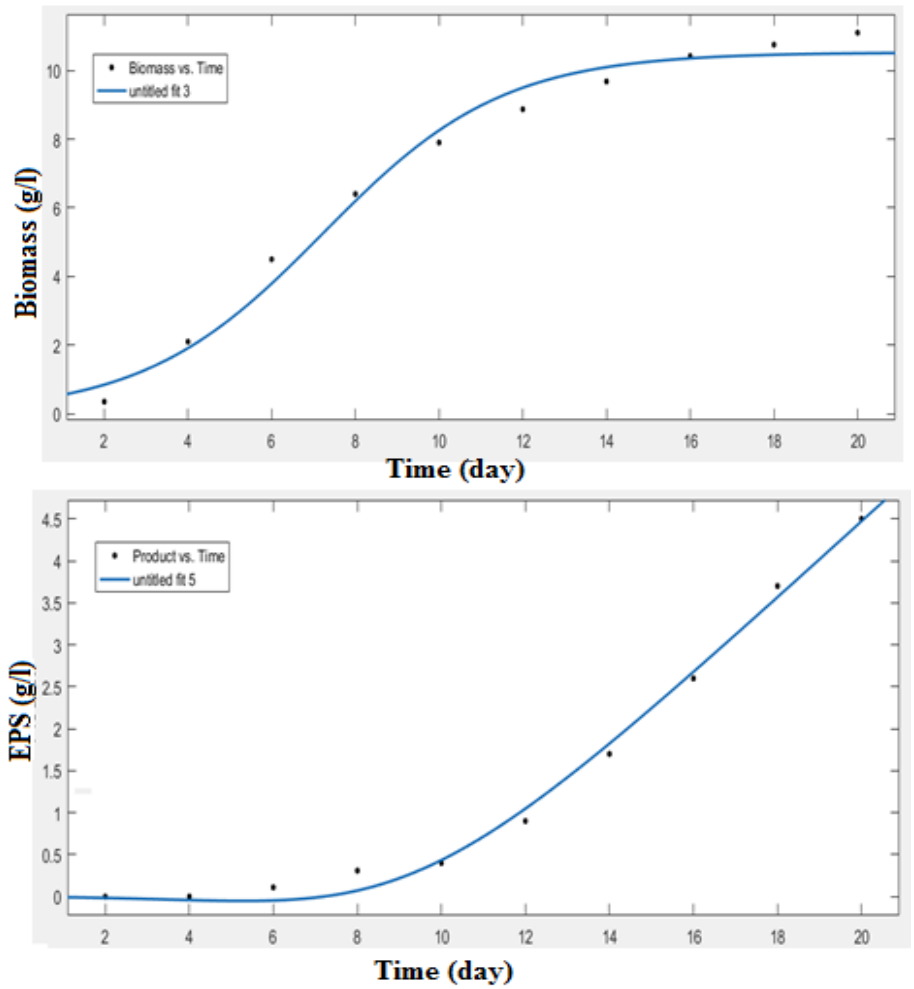

Figure 3. Temporal changes in filamentous mycelial growth (top chart) and exopolysaccharide production (bottom chart)

Fungal volatile organic compounds usually include alcohols, benzenoids, hydrocarbons, aldehydes, alkanes, alkenes, acids, esters, terpenes and ketones. Some of these compounds are found in all fungi and some are specific to one species. The *S. commune* releases ketones, sesquiterpene and ethanol. The fungus releases sulfur-containing compounds [31,32]. Of various fungi, Iranian *G. lucidum* and Chinese *G. lucidum* included the most significant impact on growth decrease in simultaneous aerial cocultures with Shiitake mushrooms as the biomasses reached to 3.2 and 3.5 $g.l^{-1}$, respectively ($p < 0.05$). All the bacterial strains severely decreased biomasses by inhibiting the fungal growth. *Staphylococcus aurous* and *E. coli* included the most significant effects on the fungal growth, reaching 2.44 and 2 $g.l^{-1}$, respectively ($p < 0.05$). The MVOCs produced by these two bacterial strains have been studied in several studies. Studies have shown that the quantity of production of these compounds on various days and patterns of production of these compounds in Gram-positive and Gram-negative bacteria significantly vary [33]. Antifungal effects of *S. aureus* volatile organic compounds on *A. niger*, *Candida albicans* and *Saccharomyces cerevisiae* have been reported [34,35].

Table 2. Mycelial growth kinetic coefficients and exopolysaccharide production in filamentous morphology§

C_0 ($g.l^{-1}$)	0.5
Kinetic parameter	Quantity
C_m ($g.l^{-1}$)	11.7
μ_m (day^{-1})	0.4842
P_0 ($g.l^{-1}$)	0.1
α ($g \times g^{-1}$)	1.252
β(g× (g×h) $^{-1}$)	-0.01829

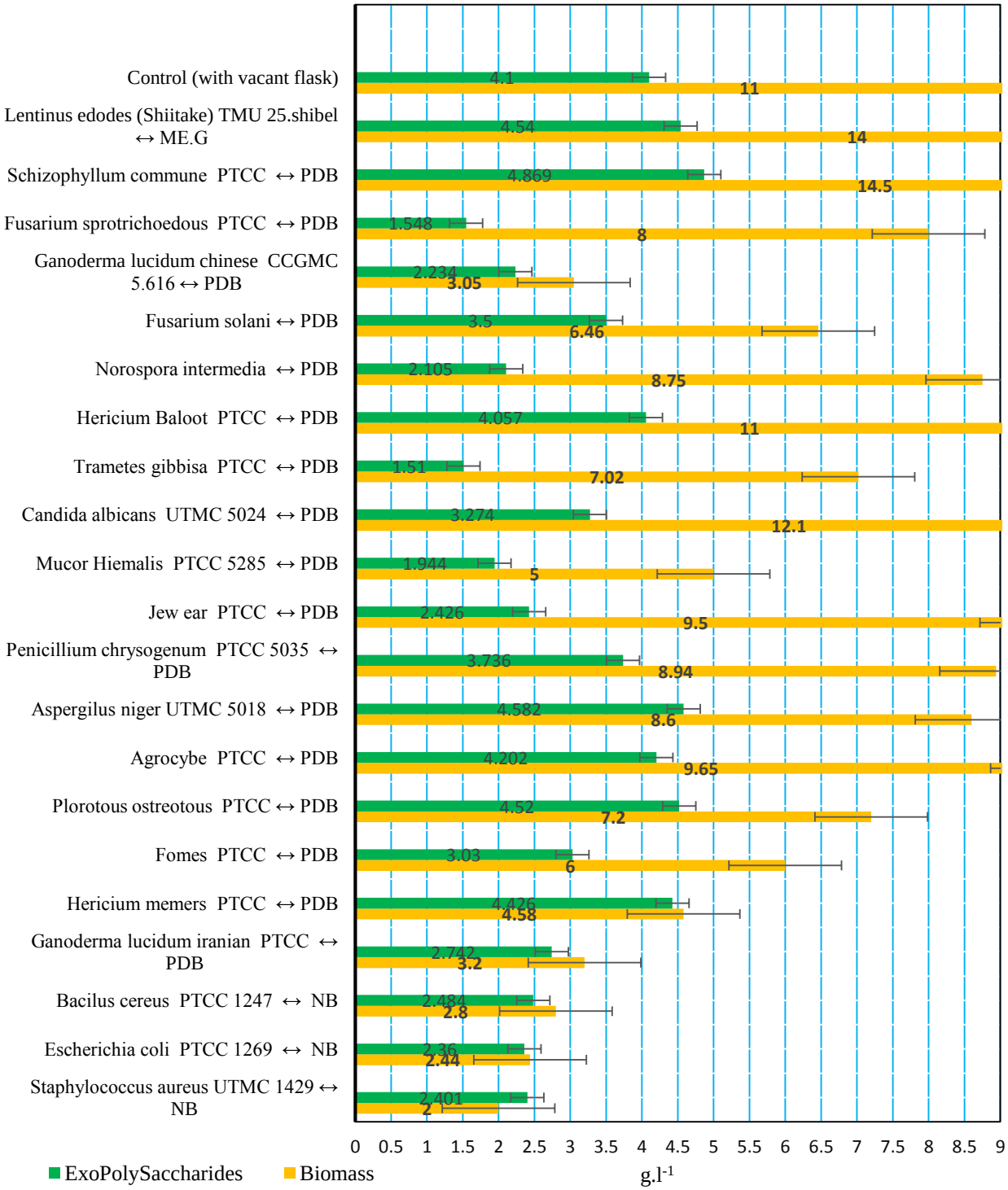

Figure 4. Graph of biomass and exopolysaccharide productions in simultaneous aerial co-cultures of Shitake with various microorganisms

Shiitake EPS production in control sample was 4.1 $g.l^{-1}$, Shiitake mushrooms produced the highest EPS in simultaneous aerial co-cultures with Shiitake, *S. commune* and *A. niger* and reached 4.54, 4.86 and 4.58 $g.l^{-1}$, respectively ($p < 0.05$). It seemed that volatile organic compounds of *S. commune* stimulated the fungi and increased production of secondary metabolites such as EPS by acting on cellular pathways. The *A. niger* in co-cultivation with Shiitake mushrooms increased production of Shiitake EPS but decreased the biomass growth of this fungus. This study as well as previous studies demonstrated that *A. niger* produced volatile organic compounds containing hydro-

carbons and alcohols [23,36]. Similar results have been achieved from the effects of simultaneous aerial co-cultures on the ability of fungi to produce various compounds [18,19,37]. In co-cultures of Shiitake mushrooms with *Fusarium sporotrichioides* and *Trametes gibbosa*, the lowest quantity of EPS was produced. Bacteria decreased production of EPS and reached 2.36 $g.l^{-1}$ in co-cultures with *E. coli*, compared to the control ($p < 0.05$).

3.5. Assessment of microbial volatile organic compounds using gas chromatography-mass spectrometry

Studies have shown that inhalation of volatile organic compounds can play antioxidant, anti-inflammatory and medicinal roles in humans. Inhalation of these compounds may play roles in decreasing fatigue, inducing relaxation and improving moods [38,39]. The present study seems to be the first study to investigate effects of MVOCs in form of simultaneous aerial cultures on Shiitake mushrooms. In analysis of MVOCs in co-cultures of Shiitake mushrooms with *A. niger*, various alcohols (Figure 5) were produced; similar to those in databases of MVOCs [27,28]. In co-cultures of Shiitake mushrooms with *S. commune*, large quantities of various alcohols (Figure 6), especially ethanol and sulfur compounds such as CS_2, were produced, similar to those in databases of MVOCs. As reported previously, presence of sulfur compounds at low concentrations can increase growth of microorganisms [40].

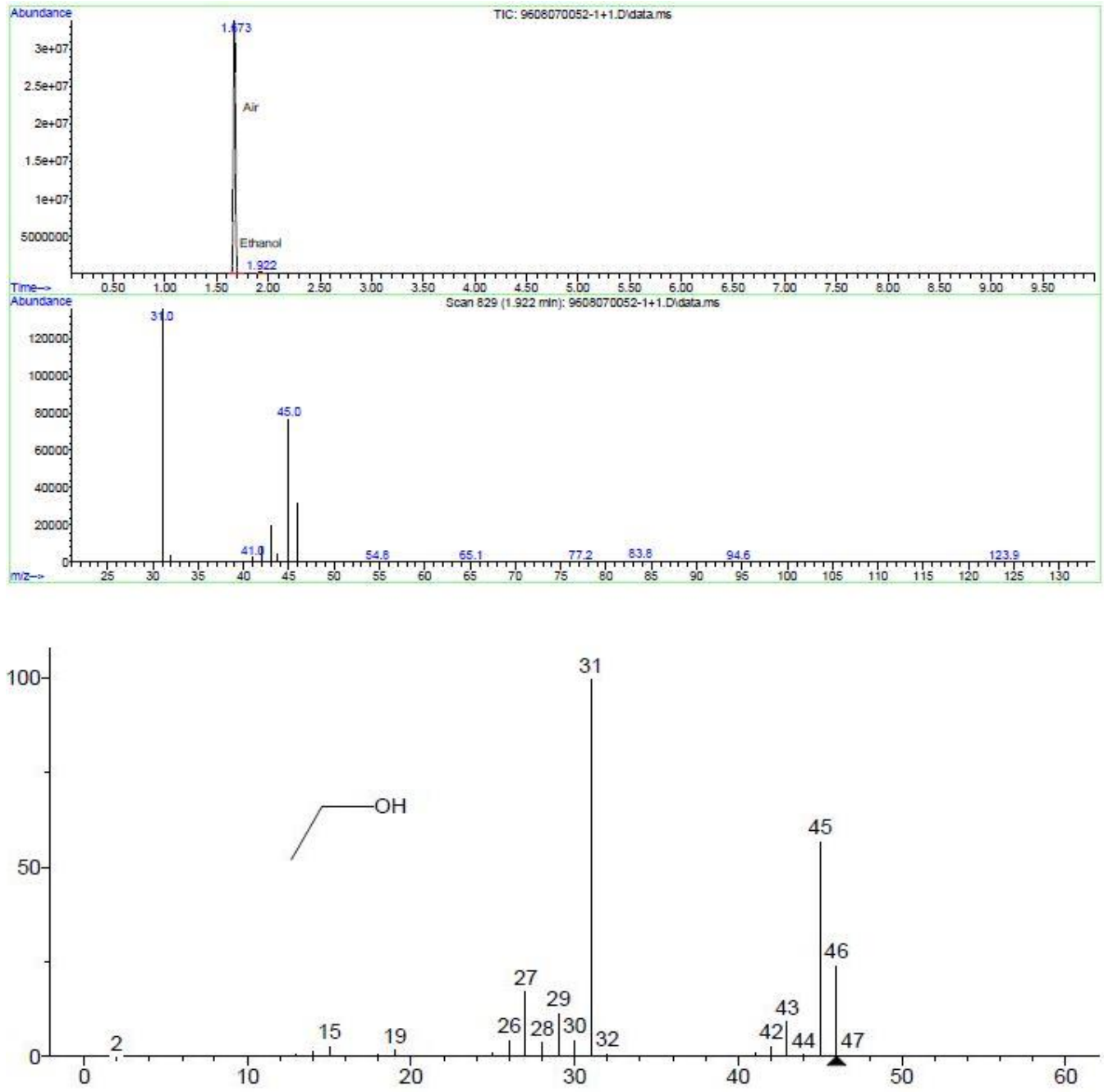

Figure 5. Data from gas chromatography-mass spectrometry of Shiitake and *Aspergillus niger*, showing presence of large quantities of ethanol and other alcohols

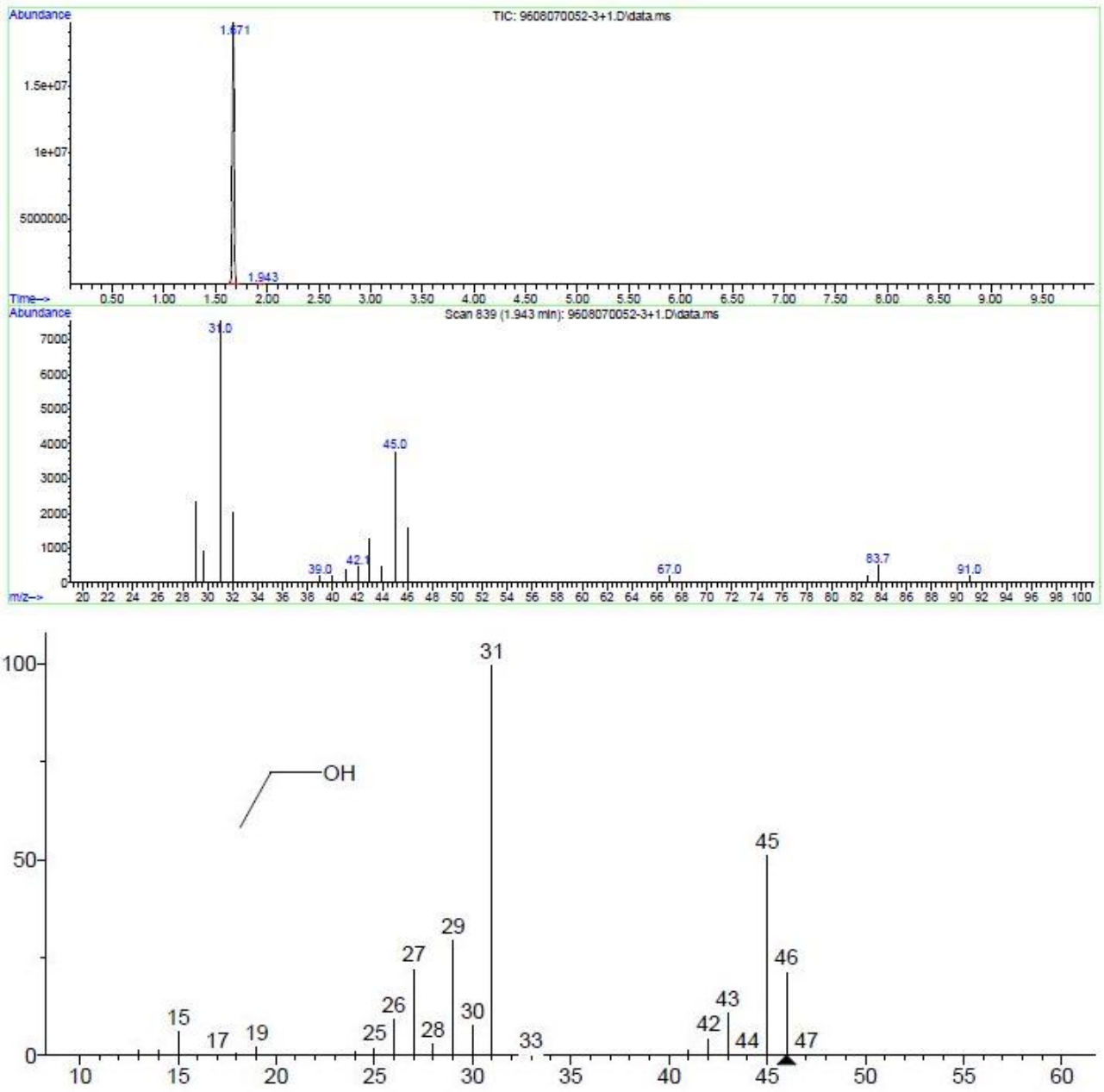

Figure 6. Data from gas chromatography-mass spectrometry analysis of Shiitake and *Schizophyllum commune*, showing presence of large quantities of ethanol and other alcohols

4. Conclusion

In this study, appropriate culture media for Shiitake growth included MEB.G media for submerged cultures and MEA.G media for solid cultures. Biomass and EPS productions included 11 and 4 $g.l^{-1}$ in these culture media. Kinetic growth investigation showed that the filamentous morphology included higher production rates with maximum specific growth rates due to higher surface-to-volume ratios, compared to that the pellet morphology did. To increase Shiitake polysaccharide production, aerial co-cultures were used. Results showed that *A. niger* and *S. commune* co-cultures with Shiitake mushrooms increased biomass and EPS productions in the mushrooms. EPS production reached 4.58 and 4.86 $g.l^{-1}$, respectively. Therefore, the present study has clearly shown that MVOCs from various microorganisms can affect growth and production of metabolites in Shiitake fungi with inductive effects and functions as elicitors.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

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مدل‌سازی کینتیک رشد و تولید پلی ساکارید خارج سلولی میسلیوم توسط *لنتینوس ادودس* (قارچ خوراکی شیتاکه)

اشرف السادات حاتمیان زارمی[1*]، زهرا تشرفی[1]،هاله الوندی[1]، محمد برشان تشنیزی[1]، بهمن ابراهیمی حسین زاده[1]، زهرا بیگم مختاری حسینی[2]

۱- گروه مهندسی علوم زیستی، دانشکده علوم و فنون نوین، دانشگاه تهران، تهران، ایران.
۲- گروه مهندسی شیمی، دانشکده شیمی و مهندسی شیمی، دانشگاه حکیم سبزواری، سبزوار، ایران.

چکیده

سابقه و هدف: *لنتینوس ادودس* (شیتاکه) منبعی غنی از متابولیت‌های ثانویه از جمله پلی‌ساکاریدهای خارج‌سلولی است. این ترکیبات توان سیستم ایمنی را افزایش می‌دهند و در پیشگیری و درمان بسیاری از بیماری‌ها، از جمله سرطان، نقش مهمی دارند. یک روش برای افزایش تولید پلی‌ساکاریدها، استفاده از محرک‌ها[1] می‌باشد. مثال‌هایی از این محرک‌ها، شامل ترکیبات آلی فرار میکروبی، است، که در کشت همزمان میکروارگانیسم‌ها تولید می‌شوند. هدف تحقیق حاضر، بررسی اثرات این ترکیبات بر تولید پلی‌ساکاریدهای شیتاکه بود.

مواد و روش ها: به‌منظور کاهش زمان کشت، شیتاکه در چهار محیط کشت، شامل ۱، مایع دکستروز سیب‌زمینی، ۲، مخلوط مایع دکستروز سیب‌زمینی و د-گلوکز، ۳، مایع عصاره مالت و ۴، مخلوط مایع عصاره مالت و د-گلوکز کشت داده شد. پس از انتخاب محیط کشت مناسب، منحنی رشد قارچ، کینتیک رشد، ریخت‌شناسی[2] فرم‌های پلت و رشته‌ای بررسی شد. روش نوین کشت همزمان هوایی برای افزایش تولید پلی‌ساکاریدهای شیتاکه، که به‌عنوان یک محرک توسط ترکیبات آلی فرار میکروبی سایر میکروارگانیسم‌ها عمل کرد، مورد استفاده قرار گرفت. ترکیبات فرار میکروبی با استفاده از کروماتوگرافی گازی جرمی مورد آنالیز قرار گرفتند.

یافته‌ها و نتیجه‌گیری: محیط کشت عصاره مالت حاوی گلوکز برای کشت های جامد و غوطه‌ور شیتاکه انتخاب شد و طول دوره رشد را به ۱۸ روز رسید. تولید زی‌توده[3] شیتاکه $g.l^{-1}$ ۱۱ بود. فرم‌های رشته‌ای به‌دلیل داشتن نسبت بیشترسطح به حجم در مقایسه با فرم‌های پلت، میزان تولید بیشتری داشتند. میزان زی‌توده و پلی‌ساکارید خارج سلولی قارچ شیتاکه در کشت همزمان هوایی با *آسپرژیلوس نایجر*، به‌ترتیب به ۱۴ و ۴ گرم بر لیتر بود. هم‌چنین زی‌توده و پلی‌ساکارید خارج سلولی در کشت همزمان هوایی با *شیزوفیلوم کومینه* به ۱۱ و ۴/۷ گرم بر لیتر رسید. ترکیبات آلی فرار میکروبی تولید شده توسط *آسپرژیلوس نایجر* و *شیزوفیلوم کومینه* در قالب کشت همزمان، به‌طور چشمگیری سبب افزایش رشد زی‌توده و تولید پلی‌ساکاریدهای خارج‌سلولی شیتاکه می‌شوند.

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

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- پلی‌ساکارید خارج سلولی
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- شیتاکه

***نویسنده مسئول**
اشرف السادات حاتمیان ضارمی
گروه مهندسی علوم زیستی، دانشکده علوم و فنون نوین، دانشگاه تهران، تهران، ایران.
تلفن: ۶۱۱۱۱۸۵۰۳-۲۱-۹۸+
پست الکترونیک:
hatamian_a@ut.ac.ir

[1] elicitors
[2] morphology
[3] biomass