Research Article

Substrate preference of Shiitake *Lentinula edodes* (Berk.) Pegler strains

Valiollah Mahdizadeh[1], Naser Safaie[1*], Mina Salehi[2] and Akbar Jahedi[1]

1. Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
2. Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

Abstract: Genus *Lentinula* comprises some of the most important edible and medicinal fungal species of the world. To compare growth rate of the strains of this genus, samples were collected from different regions of the world including Iran during 2017-2018. Investigation of the growth of 40 strains on two substrates containing malt and wheat straw extracts showed their relatively excellent performance on both media. Based on preliminary growth characteristics of the strains, 20 strains were selected to study their growth rate on natural substrate containing defined proportion of straw (40%) + sawdust (40%) + wheat bran (20%). Three strains including VM230 (UK strain), VM267 (Belgium strain) and VM353 (Japanese strain) showed high growth rate (65.25, 63.75 and 64.50 mm d^{-1}, respectively), and were identified as *Lentinula edodes* by ITS4 and ITS5 sequencing. Growth rate of the best strain (VM230) was evaluated on different substrates containing different proportions of straw, sawdust and wheat bran. The highest growth rate (58.75 mm d^{-1}) for VM230 was recorded on wheat straw (80%) + wheat bran (20%). These three strains are promising for commercial production of Shiitake.

Keywords: medicinal fungus, growth, taxonomy, malt, straw

Introduction

Genus *Lentinula* Earle, Bull. New York Bot. Gard. 5: 416 (1909) is a small genus of woody agarics in *Marasmiaceae* (*Omphalotaceae*) family. Eight species have been identified and recorded in this genus so far. Several taxonomic studies of this genus have been conducted in various countries (Pegler, 1983; Guzmán *et al.*, 1997; Nicholson *et al.*, 1997; Hibbett *et al.*, 1998; Thon, 1998; Royse and Thon, 1999; Mata-Greenwood and Petersen, 2000; Mata *et al.*, 2001; Mata, 2002; Nicholson *et al.*, 2009; Tham *et al.*, 2012; Mata and Mishra, 2015; George *et al.*, 2016). *Lentinula edodes* (Berk.) Pegler is known as Shiitake and is used for the increment of immune system, the treatment of various diseases such as cancer, AIDS, allergies, fungal infections, frequent colds and the decrement of cholesterol level (Jong and Birmingham, 1993; Fang *et al.*, 2006).

Various studies have been conducted in different countries (Royse, 1985; Royse and Bahler, 1986; Royse *et al.*, 1990; Worrall and Yang, 1992; Royse, 1996; Kilpatrick *et al.*, 2000; Royse and Sanchez-Vazquez, 2001; Curvetto *et al.*, 2002; Philippoussis *et al.*, 2003; Royse and Sanchez-Vazquez, 2003;

Handling Editor: Vahe Minassian

[*] **Corresponding author:** nsafaie@modares.ac.ir
Received: 06 September 2020, Accepted: 29 September 2020
Published online: 17 October 2020

Özçelik and Pekşen, 2007; Royse and Sanchez, 2007; Eira *et al.*, 2005; Zweigle, 2010; Alemu, 2015; Zied *et al.*, 2016) including Iran (Razeghi *et al.*, 2009; Razeghi-Yadak *et al.*, 2010; Biranvand *et al.*, 2012; Ranjbar and Olfati, 2017) regarding the quality comparison of strains as well as substrates.

Despite the great commercial importance of *Lentinula* (Capelli, 1984; Zhao *et al.*, 2011), there is no report of it occurrence in Iran. There were questions that should be answered; is there shiitake in Iran? What is the taxonomic status of shiitake in Iran? Is it possible to commercially produce shiitake with domestic facilities and resources? What is the best way to increase the biological efficiency of this fungus? To answer these questions, comprehensive sampling and collecting were conducted from different countries and compared the efficiency of different strains. Due to the effect of culture medium on the growth (Salehi *et al.,* 2017), the growth of different strains was likewise investigated on different media and substrates. The previous studies have shown that different strains of shiitake display different genetic diversity and efficiency. Therefore, collecting and identifying shiitake strains was conducted to conserve biological resources and evaluate existing strains of this fungus in Iran, and to introduce commercial valuable strains of shiitake.

Materials and Methods

Sampling, tissue culture, purification and maintenance of the strains

To find Iranian wild strains of shiitake, sampling was carried out in Noor, Rudsar, Amol, Ramsar, Rasht, Kalaleh, and Behshahr during 2018-2019. Other strains were collected from market and laboratories in different countries (Table 1). Specimens were placed in paper bags and transferred to the laboratory. Then the tissue was cultured on agar media containing straw and malt extract (Stamets and Chilton, 1983). Consequently, the samples were dried in a fan oven at 40 ºC, and kept in separate sealed plastic bags for further investigations. Specimens were deposited in the herbarium of Tarbiat Modares University (TMU), Tehran, Iran.

Comparison of growth rate of strains on agar medium

The strains were cultured on agar medium containing malt and straw extracts with three replications. The experiment was planned based on Randomized Complete Block Design (RCBD). The fungal growth was determined by measuring colony diameter. The growth of fungal strains was measured when they fully colonized Petri dishes, 48 h after the cultivation, and presented as growth rate (mm d^{-1}).

Growth rate of strains on defined substrate

Twenty strains were selected based on preliminary test (growth speed on agar medium, fruiting body and strand-like mycelium formations) to study growth speed on defined substrate medium [straw (40%) + sawdust (40%) + wheat bran (20%)].

Growth rate of best strain on different substrates

The best strain in terms of growth rate and fruiting body formation was cultivated on four different substrates including treatment 1: wheat straw (80%) + wheat bran (20%), treatment 2: sugarcane bagasse (80%) + wheat bran (20%), treatment 3: sawdust (80%) + wheat bran (20%), and treatment 4: wheat straw (25%) + sugarcane bagasse (25%) + sawdust (25%) + wheat bran (25%).

Genomic DNA Extraction

Genomic DNA was extracted using either microwave protocol described by Izumitsu *et al.* (2012) or the modified method suggested by Mahdizadeh *et al*. (2016). After crushing all samples, they were transferred to water bath at 65 °C for 10–15 min. Then the tubes were centrifuged at 10000 rpm for 10 min. The supernatants were transferred to new tubes and

kept at 37 °C for 30–60 min, after adding RNAse; DNA concentration was measured with a Nanodrop spectrophotometer and diluted to 50 ng μl^{-1} in water.

Polymerase chain reaction (PCR) and sequencing

PCR was performed following the method described by White *et al.* (1990) using universal primers ITS4 and ITS5. PCR amplification (in volume 50 µl) conditions included: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 90 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 5 min. PCR products were separated in a 1.5% agarose gel. The gel was stained with ethidium bromide (0.50 µg ml^{-1}) and visualized under UV to confirm DNA amplification. Sequencing was performed on ABI Prism Genetic analyzer (Applied Biosystems) at Beckman Coulter Genomics, England or ABI PRISM, 3730XL Analyzer at Macrogen, Korea. The phylogenetic analysis was made as described previously (Salehi *et al.*, 2018; 2019).

Statistical analysis

Statistical analysis of growth rate data was performed by general linear model (GLM) in SAS.9.1.3.Service Pack 2. Means comparisons were performed by Duncan's multiple range test at $p < 0.05$.

Results

Shiitake strains were collected from four countries in four continents. It is noteworthy that 19.0, 11.9, 7.1, 7.1, 4.7, 4.7, 4.7, 4.7, 4.7, 2.4, 2.4, 2.4, 2.4, 2.4 and 2.4% of strains were collected from China, Malaysia, Japan, Belgium, UK, Spain, Kazakhstan, Germany, Canada, England, France, Hong Kong, Indonesia, Singapore and Vietnam, respectively (Table 1). Also, 17.7% of strains were received from Iranian mushroom seed production companies (Table 1). Indeed, the most frequent strains were from China (Table 1).

Table 1 List of 42 shiitake strains used in present study.

Formal code	Country	Local name	Date
VM250	Belgium	sh-bel	1-Aug-11
VM344	Bulgaria	sh-bul1	20-Nov-17
VM345	Bulgaria	sh-bul2	20-Nov-17
VM349	Canada	sh-canada1	15-Mar-18
VM350	Canada	sh-canada2	15-Mar-18
VM316	China	sh-zhengzhu	06-Dec-17
VM317	China	sh-biang	06-Dec-17
VM319	China	sh-gracy	06-Dec-17
VM320	China	sh-pek1	06-Dec-17
VM321	China	sh-pek2	06-Dec-17
VM322	China	sh-pek3	06-Dec-17
VM323	China	sh-pek4	06-Dec-17
VM324	China	sh-pek5	06-Dec-17
VM230	England	sh-eng	30-Nov-15
VM347	France	sh-fr	05-Oct-17
VM342	Germany	povel-german1	20-Nov-17
VM343	Germany	povel-german2	20-Nov-17
VM339	Hong Kong	sh-hongkong	28-Dec-17
VM278	Indonesia	sh-sugi	26-Apr-15
VM162-1	Iran*	sh1	08-Oct-13
VM162-2	Iran*	sh2	12-May-15
VM216	Iran*	sh3	06-Sep-15
VM224	Iran*	sh-sp	06-Sep-15
VM260	Iran*	sh4	08-Oct-13
VM264	Iran*	sh-h	08-Oct-13
VM267	Iran*	sh-rbl	01-Aug-11
VM351	Japan	sh-jp1	29-Apr-18
VM352	Japan	sh-jp2	29-Apr-18
VM353	Japan	sh-jp3	29-Apr-18
VM312	Kazakhstan	sh-gaz1	06-Dec-17
VM313	Kazakhstan	sh-gaz2	06-Dec-17
VM268	Malaysia	sh-rory	03-Apr-17
VM281	Malaysia	shi1-chris	22-May-17
VM282	Malaysia	shi2-chris	22-May-17
VM308	Malaysia	sh-pinang	12-Oct-17
VM309	Malaysia	sh-kuala	10-Dec-17
VM298	Singapour	sh-sngpr	22-May-17
VM335	Spain	sh-spain1	24-Dec-17
VM336	Spain	sh-spain2	24-Dec-17
VM337	UK	sh-uk1	01-Jan-18
VM338	UK	sh-uk2	01-Jan-18
VM348	Vietnam	sh-vietnam	24-Feb-18

* Supplied from Iranian mushroom seed production companies, but its origin has not been determined.

Effects of agar medium containing malt and straw extract on the growth of different strains
Analysis of variance (ANOVA) showed that the main effects of factors “substrate type and strain” and also their reciprocal interactions on growth were highly significant ($p < 0.01$) (Table 2), suggesting that substrate type variously affected growth of each strain. Therefore, substrate type was further scrutinized on each strain to precisely analyze these significant interactions.

Table 2 Analysis of variance for the effects of agar media containing malt and straw extracts and strains on growth.

Source of variation	Degree of freedom	Growth
Block	2	1.8*
Medium (A)	1	15.0**
Strain (B)	39	7.4**
A × B	39	1.4**
Error	158	0.5

* and ** indicate significant difference at p < 0.05 and p < 0.01, respectively.

Effects of substrate type on the growth of each strain
According to Student’s t-test (Table 3), the growth of 80% strains (VM162-1, VM162-2, VM216, VM230, VM260, VM267, VM278, VM281, VM282, VM298, VM308, VM309, VM313, VM316, VM317, VM319, VM320, VM321, VM322, VM335, VM336, VM337, VM338, VM342, VM343, VM344, VM345, VM347, VM348, VM349, VM351 and VM353) displayed no statistically significant difference on agar media containing malt and straw extracts. Accordingly, the growth of 12.5% strains (VM224, VM250, VM264, VM268 and VM53) on agar medium containing malt extract was significantly higher than that containing straw extract (Table 3). Also, 7.5% of the strains (VM312, VM339 and VM350) exhibited significantly higher growth on agar media containing straw extract than that containing malt extracts (Table 3).

Average growth rate of 40 strains is presented in Table 4. The mean comparison showed that VM224, VM338, VM264, VM216, VM268, VM353, VM267, VM352, VM337, VM162-2, VM250, VM230, VM351, VM344, VM348, VM260, VM343, VM347, VM342 and VM336 strains displayed significantly higher growth as compared to other strains on agar media containing malt extract (Table 4). Accordingly, VM224, VM338, VM264, VM216, VM268, VM353, VM267, VM352, VM337, VM162-2, VM250, VM230, VM351, VM344, VM348, VM260, VM347, VM342, VM336, VM313, VM345, VM322, VM162-1, VM320, VM282, VM308 and VM312 exhibited significantly higher growth as compared to other strains on agar media containing straw extract (Table 4).

Comparison of growth rate of strains on substrate medium
Twenty strains were selected based on preliminary test (growth speed on agar medium, fruiting body formation and strand-like mycelium) to study growth speed on substrate medium. According to analysis of variance, there was a significant difference between the strains in terms of mycelial growth rate (Data not shown). The mean comparison showed that VM230, VM353, VM267, VM322, VM338, VM224, VM312, VM162-2, VM308, VM260, VM282, VM298, VM264, VM336, VM319 and VM268 strains displayed no significant difference in growth, and their growth was significantly higher than VM216, VM343, VM250 and VM345 strains on defined substrate (straw (40%) + sawdust (40%) + wheat bran (20%)) (Table 5).

Identification of VM230, VM353 and vm267 strains
As shown in Table 5, strains including VM230 (UK strain), VM267 (Belgium strain) and VM353 (Japanese strain) displayed high growth (65.25, 63.75 and 64.50 mm d-1, respectively) among 20 strains evaluated on defined substrate. These strains were identified as *Lentinula edodes* (Fig. 1). ITS1-5.8s-ITS2 sequences obtained from VM230, VM267 and VM353 were deposited in GenBank database under accession numbers MW021134, MW021135 and MW021136, respectively.

Comparison of qualitative properties of strains VM162-2, VM216, VM224, VM230, VM250, VM260, VM264, VM267, VM268, VM282, VM298, VM308, VM312, VM319, VM322, VM336, VM338, VM343, VM343 andVM345 strains were selected for substrate test. Selection was carried out based on one or more pin head formation on any replicate of both agar medium tests. The strains were grouped in terms of quality according to Table 6 .

Table 3 Results of t-test and descriptive statistics for equality of growth rate of the strains on agar media containing malt and straw extracts.

Strain	Growth rate (Mean ± SE) (mm d^{-1})				t-test	df
	Malt extract	n	Straw extract	n		
VM162-1	20.17 ± 0.08	3	20.00 ± 0.00	3	2.00^{ns}	4
VM162-2	21.33 ± 0.36	3	20.17± 0.22	3	2.75^{ns}	4
VM216	21.50 ± 0.29	3	20.5 ± 0.38	3	2.09 ^{ns}	4
VM224	**22.08 ± 0.30**	3	20.50 ± 0.38	3	3.26*	4
VM230	21.08 ± 0.60	3	20.083 ± 0.17	3	1.60^{ns}	4
VM250	**21.17 ± 0.17**	3	20.33 ± 0.08	3	4.47*	4
VM260	20.83 ± 0.17	3	20.92 ± 0.08	3	-0.45^{ns}	4
VM264	**21.75 ± 0.00**	3	20.17 ± 0.17	3	9.50**	4
VM267	21.42 ± 0.42	3	21.00 ± 0.00	3	1.00^{ns}	4
VM268	**21.50 ± 0.29**	3	20.50 ± 0.14	3	3.10*	4
VM278	18.25 ± 0.43	3	17.42 ± 0.30	3	1.58^{ns}	4
VM281	17.67 ± 0.42	3	16.75 ± 0.29	3	1.81^{ns}	4
VM282	20.08 ± 0.22	3	21.08 ± 0.42	3	-2.12^{ns}	4
VM298	19.33 ± 0.33	3	18.33 ± 0.44	3	1.81^{ns}	4
VM308	20.08 ± 0.36	3	20.00 ± 0.14	3	0.21^{ns}	4
VM309	19.25 ± 0.29	3	18.83 ± 0.42	3	0.82^{ns}	4
VM312	19.67 ± 0.22	3	**21.5 ± 0.14**	3	-6.96**	4
VM313	20.42 ± 0.17	3	20.00 ± 0.14	3	1.89^{ns}	4
VM316	20.42 ± 0.30	3	16.42 ± 1.80	3	2.19^{ns}	4
VM317	19.50 ± 0.14	3	19.33 ± 0.30	3	0.50^{ns}	4
VM319	19.75 ± 0.29	3	19.67 ± 0.93	3	0.09^{ns}	4
VM320	20.17 ± 0.93	3	20.17 ± 0.22	3	0.00^{ns}	4
VM321	20.33 ± 0.17	3	19.92 ± 0.22	3	1.51^{ns}	4
VM322	20.25 ± 0.43	3	20.42 ± 0.17	3	-0.36^{ns}	4
VM335	19.33 ± 0.46	3	19.83 ± 0.17	3	-1.01^{ns}	4
VM336	20.67 ± 0.68	3	20.08 ± 0.08	3	0.85^{ns}	4
VM337	21.33 ± 0.36	3	20.67 ± 0.08	3	1.79^{ns}	4
VM338	22.00 ± 0.43	3	20.17 ± 0.22	3	3.86^{ns}	4
VM339	19.25 ± 0.00	3	**19.92 ± 0.22**	3	-3.02*	4
VM342	20.67 ± 0.46	3	20.00 ± 0.25	3	1.26^{ns}	4
VM343	20.75 ± 0.52	3	19.33 ± 0.36	3	2.23^{ns}	4
VM344	21.00 ± 0.43	3	20.00 ± 0.58	3	1.39^{ns}	4
VM345	20.33 ± 0.33	3	20.58 ± 0.46	3	-0.44^{ns}	4
VM347	20.67 ± 0.17	3	20.33 ± 0.08	3	1.79^{ns}	4
VM348	20.92 ± 1.01	3	20.08 ± 0.22	3	0.80^{ns}	4
VM349	16.58 ± 0.58	3	17.50 ± 0.00	3	-1.57^{ns}	4
VM350	16.92 ± 0.17	3	**18.08 ± 0.30**	3	-3.40*	4
VM351	21.00 ± 0.43	3	20.92 ± 0.30	3	0.16^{ns}	4
VM352	**21.42 ± 0.22**	3	20.25 ± 0.14	3	4.43*	4
VM353	21.50 ± 0.14	3	20.58 ± 0.30	3	2.75^{ns}	4

* and ** indicate significant difference at $p < 0.05$ and $p < 0.01$ respectively. ns indicate non-significant.

Table 4 Comparison of average growth rate (mm d-1) of 40 strains on agar media containing malt and straw extracts.

Strains	Malt extract agar	Straw extract agar
VM224	22.08a	20.50A-C
VM338	22.00a	20.17A-D
VM264	21.75ab	20.17A-D
VM216	21.50a-c	20.50A-C
VM268	21.50a-c	20.50A-C
VM353	21.50a-c	20.58A-C
VM267	21.42a-c	21.00AB
VM352	21.42a-c	20.25A-D
VM337	21.33a-c	20.67A-C
VM162-2	21.33a-c	20.17A-D
VM250	21.17a-d	20.33A-D
VM230	21.08a-d	20.08A-D
VM351	21.00a-e	20.92AB
VM344	21.00a-e	20.00A-D
VM348	20.92a-e	20.08A-D
VM260	20.83a-f	20.92AB
VM343	20.75a-g	19.33C-F
VM347	20.67a-g	20.33A-D
VM342	20.67a-g	20.00A-D
VM336	20.67a-g	20.08A-D
VM316	20.42b-g	16.42I
VM313	20.42b-g	20.00A-D
VM345	20.33b-g	20.58A-C
VM321	20.33b-g	19.92B-D
VM322	20.25b-g	20.42A-C
VM162-1	20.17c-g	20A-D
VM320	20.17c-g	20.17A-D
VM282	20.08c-g	21.08AB
VM308	20.08c-g	20.00A-D
VM319	19.75d-h	19.67B-E
VM312	19.67d-h	21.50A
VM317	19.50e-h	19.33C-F
VM335	19.33f-h	19.83B-E
VM298	19.33f-h	18.33E-G
VM339	19.25gh	19.92B-D
VM309	19.25gh	18.83D-G
VM278	18.25hi	17.42G-I
VM281	17.66ij	16.75HI
VM350	16.92ij	18.08F-H
VM349	16.508j	17.50G-I

Means followed by the same letters in each column are not significantly different (Duncan's multiple range test at $p < 0.05$).

Table 5 Average growth rate of 20 selected strains on defined substrate: straw (40%) + sawdust (40%) + wheat bran (20%).

Strain	Growth rate (mm d^{-1})	Strain	Growth rate (mm d-1)
VM230	65.25a	VM282	60.33a-d
VM353	64.50a	VM298	58.67a-d
VM267	63.75ab	VM264	58.42a-d
VM322	62.583ab	VM336	57.92a-d
VM338	62.50ab	VM319	57.00a-d
VM224	62.50ab	VM268	56.67a-d
VM312	61.42a-c	VM216	55.083b-e
VM162-2	61.25a-d	VM343	53.00c-e
VM308	61.08a-d	VM250	51.92de
VM260	60.67a-d	VM345	47.08e

Means followed by the same letters in a column are not significantly different (Duncan's multiple range test at $p < 0.05$).

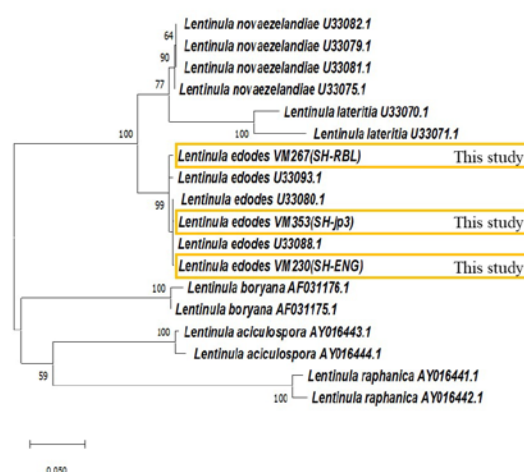

Figure 1 Molecular identification of VM230, VM353 and VM267 strains based on the analysis of the sequences of actin gene. The tree was rooted to *Lentinula raphanica*.

Comparison of cultivation substrates

Growth rate of the most promising strain (VM230) was evaluated on different substrates containing different proportion of straw, sawdust and wheat bran. The highest growth rate (58.75 mm d^{-1}) of VM230 was recorded on wheat straw (80%) + wheat bran (20%) (Fig. 2).

Table 6 Grouping of *Lentinula edodes* strains based on qualitative characteristics.

Strains	Qualitative characteristics
VM230, VM308, VM319, VM343, VM345, VM298	Fruiting body formation on agar medium
VM230, VM308, VM319, VM343, VM353, VM312, VM267, VM162-2, VM260	Fruiting body formation on substrate
VM224, VM338, VM264, VM216, VM268, VM353, VM267, VM352, VM337, VM162-2, VM250	Best growth rate on malt agar medium
VM312, VM282, VM267, VM351, VM260, VM337, VM353, VM345, VM268, VM224, VM216, VM322	Best Growth rate on straw extract agar medium
VM230, VM353, VM267, VM322, VM338, VM224, VM312, VM162-2	Strains with the highest growth rate on substrate

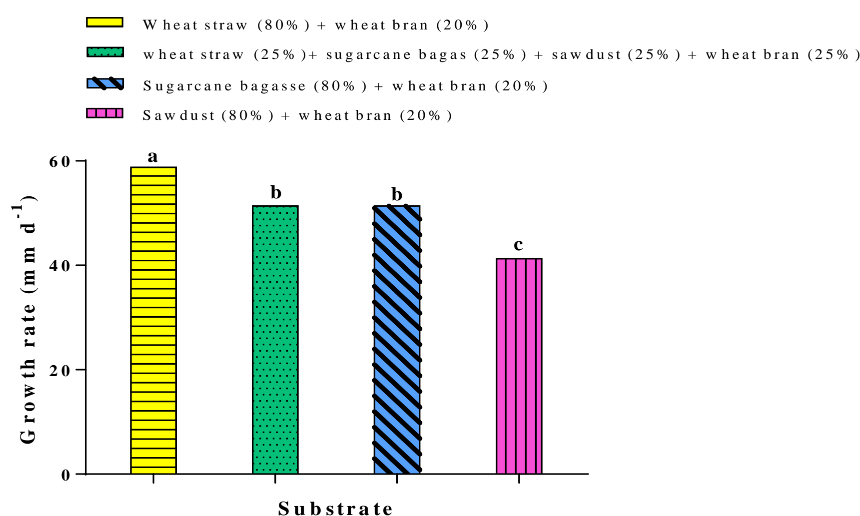

Figure 2 Average growth rate of *Lentinula edodes* strain VM230 on different substrates.

Discussion

In this study, 42 strains of *Lentinula* were collected, purified, and finally 40 strains were maintained. To our knowledge, this project has been the largest study regarding the number of strains of this fungus in the world so far. The largest collection of this fungus was added to the country's biodiversity culture collection through this study.

The growth rate of the strains was assessed on agar medium containing malt and straw extracts. Most of the strains showed good growth on both agar media containing malt and straw extracts. Formerly there was no study on growth rate of shiitake on agar media containing malt and straw extracts. In one study, agar medium containing sorghum flour, millet flour, wheat flour, potato flour was compared, and there was a difference between different culture media, the medium containing sorghum and potato displayed the highest and lowest growth rate, respectively (Chittaragi *et al.*, 2018). The effect of a combination of culture medium, acidity and temperature on the growth rate and weight of shiitake mycelium was investigated in solid and liquid culture media in Iran (Razeghi *et al.*, 2009). Their results showed the significant effect of culture medium, acidity and temperature on mycelia growth rate.

According to our results, there was a significant difference between growth rates of the strains. Therefore, strains with a higher

growth rate have a better potential for cultivation. These strains have the higher competitiveness and less contamination of competing fungi. Also their growth period is reduced, and production will be commercially beneficial (Siwulski *et al.*, 2009; Lahijani and Farsi, 2017).

Our results showed that growth rate of some of strains were significantly different on different agar media. Therefore, different culture media should be investigated and the best medium for each strain should be selected.

Pinning feature on agar medium or early pinning on substrate medium was one of the distinguishing features among the strains. This feature was considered as a good one and strains can be selected based on it for future analysis.

After comparing growth rate on two culture media and their morphological characteristics, twenty strains were selected to compare their growth rate on defined substrate medium (straw (40%) + sawdust (40%) + wheat bran (20%))

Growth rate and fruiting body formation of the best strain was studied in order to find the most suitable cultivation substrate. There was significant difference among treatments as wheat straw-based substrate displayed the highest growth speed (Table 5).

Similar to our results, Zied *et al.* (2016) compared genetic diversity and fungal production (yield, number and weight of fruiting body) using different media and cultivation conditions, and their results showed that the yield and fruiting time of the strains differed significantly under different cultivation conditions and media (Zied *et al.*, 2016).

Conclusion

This research evaluated the different strains of Shiitake in terms of growth rate on different media and substrates for the first time. VM230, VM267 and VM353 strains displayed high growth rate, and were identified as *Lentinula edodes*. The highest growth rate of VM230 was recorded on wheat straw (80%) + wheat bran (20%). This research is an effective step towards the commercial production of this valuable edible and medicinal fungus in the country.

Acknowledgements

The authors acknowledge Iran National Science Foundation (INSF, No. 95004326), and Research Deputy of Tarbiat Modares University, Tehran for financial support of this research project.

References

Alemu, F. 2015. Cultivation of Shiitake Mushroom (*Lentinus edodes*) on Coffee Husk at Dilla University, Ethiopia. Journal of Food and Nutrition Sciences, 3(2): 63-70.

Biranvand, M., Alemzadeh, A. N., Mousavi, S. and Bazigar, E. 2012. Effects of substrate and substrate enrichment on physical and chemical properties of medicinal shiitake mushroom. Journal of Horticultural Science, 26(2): 162-169.

Capelli, A. 1984. Agaricus. L.: Fr. (Psalliota Fr.). Liberia editrice Bella Giovanna, Saronno, Italy.

Chittaragi, A., Kumar, A. and Singh, S. 2018. Effect of different media on mycelial growth of *lentinula edodes*. International Journal of Current Microbiology and Applied Sciences, 7(4): 2193-2198.

Curvetto, N., Figlas, D. and Delmastro, S. 2002. Sunflower seed hulls as substrate for the cultivation of shiitake mushrooms. HortTechnology, 12(4): 652-655.

Eira, F., Meirelles, W. and Paccola Meirelles, L. 2005. Shiitake production in corncob substrates. Revista Brasileira de Milho e Sorgo, 4(2): 141-148.

Fang, N., Li, Q., Yu, S., Zhang, J., He, L., Ronis, M. J. and Badger, T. M. 2006. Inhibition of growth and induction of apoptosis in human cancer cell lines by an ethyl acetate fraction from shiitake mushrooms. Journal of Alternative and Complementary Medicine, 12(2): 125-132.

George, P. L., Sripathi, V. R., Nyaku, S. T., Sharma, G. C. and Kantety, R. V. 2016. DNA-based identification of *Lentinula*

edodes strains with species-specific primers. African Journal of Biotechnology, 15(7): 191-198.

Guzmán, G., Salmones, D. and Tapia, F. 1997. Lentinula boryana, Morphological Variations, Taxonomic Position, Distribution and Relationships with *Lentinula edodes* and Related Species. Reports of the Tottori Mycological Institute, Japan.

Hibbett, D. S., Hansen, K. and Donoghue, M. J. 1998. Phylogeny and biogeography of Lentinula inferred from an expanded rDNA dataset. Mycological Research, 102(09): 1041-1049.

Izumitsu, K., Hatoh, K., Sumita, T., Kitade, Y., Morita, A., Gafur, A., Ohta, A., Kawai, M., Yamanaka, T. and Neda, H. 2012. Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. Mycoscience, 53(5): 396-401.

Jong, S. and Birmingham, J. 1993. Medicinal and therapeutic value of the shiitake mushroom. Advances in Applied Microbiology, 39: 153-153.

Kilpatrick, M., Murray, D. and Ward, F. 2000. Influence of substrate formulation and autoclave treatment on *Lentinula edodes* production. Mushroom Science, 15(2): 803-810.

Lahijani, M. and Farsi, M. 2017. Evaluation of mycelium growth rate and yield of white button mushroom isolates (*Agaricus bisporus*) in Iran. Journal of Horticulture Science, 31(1):

Mahdizadeh, V., Safaie, N., Goltapeh, E. M., Asef, M. R., Hosseini, S. M. N. and Callac, P. 2016. *Agaricus* section *Xanthodermatei* in Iran. Phytotaxa, 247(3): 181-196.

Mata-Greenwood, J. L. and Petersen, R. H. 2000. A new species of *Lentinula* (Agaricales) from Central America. Una nueva especie de Lentinula (Agaricales) de Centroamérica. Mycoscience, 41(4): 351-355.

Mata, J. L. 2002. Taxonomy and Systematics of *Lentinula*, *Gymnopus*, and *Rhodocollybia* (Agaricales, Fungi), with emphasis on oak forests of southern Costa Rica. Ph. D. Dissertation, The University of Tennessee, TN. USA.

Mata, J. L. and Mishra, N. T. 2015. Comparative study of worldwide species of genus *Lentinus* (= *Lentinula*, higher basidiomycetes) based on linear mycelium growth. International Journal of Medicinal Mushrooms, 17(5): 481-489.

Mata, J. L., Petersen, R. H. and Hughes, K. W. 2001. The genus *Lentinula* in the Americas. Mycologia, 93(6): 1102-1112.

Nicholson, M. S., Bunyard, B. A. and Royse, D. J. 1997. Phylogeny of the genus *Lentinula* based on ribosomal DNA restriction fragment length polymorphism analysis. Mycologia, 89(3): 400-407.

Nicholson, M. S., Bunyard, B. A. and Royse, D. J. 2009. Phylogenetic implications of restriction maps of the intergenic regions flanking the 5S Ribosomal RNA gene of *Lentinula* species. Fungi, 2(4): 48-57.

Özçelik, E. and Pekşen, A. 2007. Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). Bioresource Technology, 98(14): 2652-2658.

Pegler, D. 1983. The genus *Lentinula* (Tricholomataceae tribe Collybieae). Sydowia, 36: 227-239.

Philippoussis, A., Diamantopoulou, P. and Zervakis, G. 2003. Correlation of the properties of several lignocellulosic substrates to the crop performance of the shiitake mushroom *Lentinula edodes*. World Journal of Microbiology and Biotechnology, 19(6): 551-557.

Ranjbar, M. E. and Olfati, J. A., 2017. Evaluation of substrate components on shiitake mushroom properties. International Journal of Vegetable Science, 23(2): 145-150.

Razeghi-Yadak, L., Azizi, M. and Farsi, M. 2010. To Study the influence of different substrate on Shiitake mushroom fruiting (the First Production Report in Iran). Iranian Journal of Field Crops Research, 8(3): 501-507.

Razeghi, Y. L., Azizi, M., Farsi, M. and Shah, T. S. 2009. Evaluation effect of media formulation, pH and temperature on" shiitake " mycelium growth analysis on solid

and liquid culture conditions. Journal of Horticultural Sciences, 23(1): 18-26.

Royse, D., Bahler, B. and Bahler, C. 1990. Enhanced yield of shiitake by saccharide amendment of the synthetic substrate. Applied and Environmental Microbiology, 56(2): 479-482.

Royse, D. and Bahler, C. 1986. Effects of genotype, spawn run time, and substrate formulation on biological efficiency of shiitake. Applied and Environmental Microbiology, 52(6): 1425-1427.

Royse, D. J. 1985. Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. Mycologia, 77(5): 756-762.

Royse, D. J. 1996. Yield stimulation of shiitake by millet supplementation of wood chip substrate. In: Royse, D. J. (Ed.), Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products. 9-12 June, University Park, PA, pp. 277-283.

Royse, D. J. and Sanchez-Vazquez, J. E. 2001. Influence of substrate wood-chip particle size on shiitake (*Lentinula edodes*) yield. Bioresource Technology, 76(3): 229-233.

Royse, D. J. and Sanchez-Vazquez, J. E. 2003. Influence of precipitated calcium carbonate (CaCO3) on shiitake (*Lentinula edodes*) yield and mushroom size. Bioresource Technology, 90(2): 225-228.

Royse, D. J. and Sanchez, J. E. 2007. Ground wheat straw as a substitute for portions of oak wood chips used in shiitake (*Lentinula edodes*) substrate formulae. Bioresource Technology, 98(11): 2137-2141.

Royse, D. J. and Thon, M. R. 1999. Phylogeny of Shiitake of the Americas (*Lentinula boryana*) Inferred from rDNA Sequences. 3rd International Conference on Mushroom Biology and Mushroom Products and AMGA's 26th National Mushroom Industry Conference. Sydney, Australia.

Salehi, M., Moieni, A. and Safaie, N. 2017. A novel medium for enhancing callus growth of hazel (*Corylus avellana* L.). Scientific reports, 7(1): 1-9.

Salehi, M., Moieni, A. and Safaie, N. 2018. Elicitors derived from hazel (*Corylus avellana* L.) cell suspension culture enhance growth and paclitaxel production of *Epicoccum nigrum*. Scientific Report, 8(1): 1-10.

Salehi, M., Moieni, A., Safaie, N. and Farhadi, S. 2019. Elicitors derived from endophytic fungi *Chaetomium globosum* and *Paraconiothyrium brasiliense* enhance paclitaxel production in Corylus avellana cell suspension culture. Plant Cell Tissue Organ Cult. (PCTOC) 136(1): 161-171.

Siwulski, M., Sobieralski, K. and Wojniłowicz, M. 2009. Comparison of mycelium growth and yielding of selected strains of *Hericium erinaceus* (Bull. Fr.) Pers. on sawdust substrates with the glucose addition. Herba Polonica, 55(3): 266-272.

Stamets, P. and Chilton, J. S. 1983. The Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home. Agarikon press.

Tham, L. X., Chuong, N. N., Duong, P. N. and Thiem, B. H. 2012. The biogeographical speciations of shiitake *Lentinula edodes* and a new species *Lentinula platinedodes* sp. nov. found in Cat Tien, South Vietnam. Formerly, Tap Chi Sinh Hoc (Academia Journal of Biology), 33(3): 29-39.

Thon, M. R. 1998. Evolutionary Relationships among Members of The Genus *Lentinula* and other Selected Basidiomycetes Based on Beta-Tubulin and Ribosomal DNA Sequences. Ph. D. Thesis, Pennsylvania State University.

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: Innis, M. A. Gelfand, D. H. Sninsky, J. J. and White, T. J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315-322.

Worrall, J. and Yang, C. S. 1992. Shiitake and oyster mushroom production on apple pomace and sawdust. HortScience, 27(10): 1131-1133.

Zhao, R., Karunarathna, S., Raspé, O., Parra, L. A., Guinberteau, J., Moinard, M., De Kesel, A., Barroso, G., Courtecuisse, R., Hyde, K. D., Guelly, A. K., Desjardin, D. E. and Callac, P. 2011. Major clades in tropical *Agaricus*. Fungal Diversity, 51: 279-296.

Zied, D. C., Maciel, W. P., Marques, S. C., e Santos, D. M. D. S., Rinker, D. L. and Dias, E. S. 2016. Selection of strains for shiitake production in axenic substrate. World Journal of Microbiology and Biotechnology, 32(10): 168. https://doi.org/10.1007/s11274-016-2115-3

Zweigle, C. A. 2010. Pistachio byproucts [s. i. c.] as substrate for shiitake mushrooms. IndraStra Global Index (IGI), accessed October 8, 2020, http://igi.indrastra.com /items/show/138747.

ترجیح بستر رشد در استرین‌های شیتاکه *Lentinula edodes* (Berk.) Pegler

ولی‌اله مهدیزاده[1]، ناصر صفایی[1*]، مینا صالحی[2] و اکبر جاهدی[1]

۱- گروه بیماری‌شناسی گیاهی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.
۲- گروه اصلاح نباتات و بیوتکنولوژی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: nsafaie@modares.ac.ir
دریافت: ۱۶ شهریور ۱۳۹۹؛ پذیرش: ۸ مهر ۱۳۹۹

چکیده: جنس *Lentinula* مهم‌ترین گونه‌های قارچ‌های خوراکی و دارویی دنیا را در برمی‌گیرد. برای مقایسه نرخ رشد استرین‌های این جنس، نمونه‌هایی از مناطق مختلف دنیا و ایران در سال‌های ۲۰۱۷ تا ۲۰۱۸ جمع‌آوری شد. بررسی میزان رشد ۴۰ استرین روی دو سوبسترای حاوی عصاره مالت و عصاره کاه نشان داد که روی هر دو محیط رشد استرین‌ها بسیار خوب بود. براساس ویژگی‌های اولیه مربوط به رشد استرین‌ها، ۲۰ استرین برای بررسی نرخ رشد روی بسترهای طبیعی با نسبت‌های معین (۴۰٪ کاه + ۴۰٪ خاک اره +۲۰٪ سبوس گندم) انتخاب شدند. سه استرین شامل VM230، VM267 و VM353 بیش‌ترین نرخ رشد (به‌ترتیب ۶۵/۲۵، ۶۳/۷۵ و ۶۴/۵۰ میلی‌متر در روز) را نشان داد و با استفاده از تعیین توالی ناحیه ITS1-5.8S-ITS2 به‌عنوان گونه *Lentinula edodes* شناسایی شد. نرخ رشد بهترین استرین (VM230) بر روی سوبستراهای حاوی نسبت‌های مختلف کاه، خاک اره و سبوس گندم ارزیابی شد. بالاترین نرخ رشد (۵۸/۷۵ میلی‌متر در روز) VM230 روی بستره حاوی ۸۰٪ کاه گندم و ۲۰٪ سبوس گندم مشاهده شد. نتایج نشان داد که این استرین‌ها برای تولید تجاری شیتاکه امیدبخش می‌باشند.

واژگان کلیدی: قارچ دارویی، نرخ رشد، تاکسونومی، مالت، کاه