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THE MORPHOLOGICAL OBSERVATION OF MYCELIA OF SEVERAL ARMENIAN STRAINS OF MEDICINAL BRACKET FUNGUS *FOMES FOMENTARIUS* (L.) FR. (POLYPORALES, AGARICOMYCETES)

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The morphological and growth characteristics of several Armenian strains of the tinder polypore fungus *Fomes fomentarius* were screened on agar media at 25, 30, 35, 38°*C* and 40°*C*. The revealed taxonomically valuable mycelial characteristics can be used for identification of *F. fomentarius,* as well as to control the purity of cultures during their biotechnological cultivation to obtain biomass and bioactive compounds.

Keywords: *Fomes fomentarius,* mycelium, maco- and micromorphology, growth rate and temperature.

Introduction. A white-rot tinder polypore mushroom *Fomes fomentarius* (L.) Fr. (Polyporales, Agaricomycetes) is widely distributed worldwide, including all floristic regions of Armenia, where it was described on beech, oak, hornbeam, walnut, willow and other deciduous trees [1, 2]. The wound-healing and anti-inflammatory properties of this fungus were known from ancient times [3]. In traditional medicine of Asian countries, the tinder polypore was also used as an anticancer and homeostatic agent [4]. The antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, immunomodulating, hypoglycemic, hypolipidemic and antioxidant effects were also reported, due to bioactive compounds (phenolics, flavonoids, polysaccharides, triterpenoids, ketons, etc.) produced by *F. fomentarius* [5–9].

For biotechnological cultivation of Agaricomycetes mushrooms, the optimal growth conditions (temperature, pH, nutrient medium, etc.) and the control of purity of cultures are required to obtain mycelial biomass and bioactive compounds. Moreover, several taxonomically valuable mycelial characteristics can be used for identification of fungal cultures during their vegetative growth followed by an extensive screening for perspective strains [10–16].

In the current study, morphological and growth characteristics of several Armenian strains of *F. fomentarius* at different culture conditions are presented.

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Materials and Methods.

Fungal Collection. Fruiting bodies of *F. fomentarius* were collected in Ijevan and Aparan floristic regions of Armenia on beech, hornbeam, walnut and willow trees during years 2014–2016 (Tab. 1). After morphological identification of basidiomes, 6 dikaryotic cultures were isolated [17, 18]. The cultures are preserved in the Fungal Culture Collection of the Laboratory of Fungal Biology and Biotechnology, Yerevan State University (FCC-YSU) under the catalogue numbers [16].

Table 1

Studied Armenian strains of Fomes fomentarius

Catalogue number	Strain	Origin and date of isolation	Wood substrate
5206	Ff/8	Berdavan village, IFR, 2014	*Juglans regia*
5207	Ff/11	Berdavan village, IFR, 2014	*Fagus* tree
5210	Ff/14	Hankavan village, AFR 2014	Deciduous tree
5216	Ff/16	Solak village, AFR, 2015	*Salix alba* stump
5218	Ff/18	Marmarik village, AFR, 2015	Deciduous tree
5223	Ff/26	Near Lake Parz, IFR, 2016	*Carpinus* tree

Note: IFR – Ijevan Floristic Region; AFR – Aparan Floristic Region.

The Study of Morphological and Growth Characteristics of Mycelia. Morphological observation of mycelial colonies and determination of their growth rate (GR) were performed on 1.5% malt-extract agar (MEA) and potato-dextrose agar (PDA) media (pH 6) after inoculation by 5 mm^3 inocula into the center of 90 *mm* Petri dishes (25 *mL* medium in each) and incubation in the dark at 25, 30, 35, 38 and 40°*C* during 3 weeks [11]. After incubation the plates were transferred into the room temperature 20 ± 2°*C* under day/night regime in humid condition to observe *in vitro* fruiting ability during 1–1.5 months. The texture and pigmentation of colonies were described using Stalpers' scale [12]. Mycelial GR was calculated according to the formula $GR=\Delta d/\Delta t$, where Δd is the difference between diameters of colonies (*mm*) during Δt time (days). The average growth rate (GR_{avr}) was calculated from GR data obtained during 6 days of growth. The observation of micromorphological characteristics of mycelia was performed by a previously described slide method using an Omano OM157-T Trinocular microscope with 15 × 40 ocular / objective [19–21]. All photos were taken with an OptixCam Summit OCS 1.3 MP digital microscope camera with the OC View 7 software program (Version 7.1). The results were statistically analyzed using the SLOPE algorithm (Microsoft Excel, Microsoft Corp., Redmond, WA, USA) and expressed as mean ± SD.

Results and Discussion.

Morphological Observation of Cultures. At 25°*C* on MEA the strains of *F. fomentarius* formed white, woolly cottony, later felt-suede, white, creamy-cinnamon or cinnamon-brown colonies with unchanged, yellow-brown or dark brown agar (Fig. 1). The tested strains grow relatively slowly on PDA and form denser colonies with intense brown pigmentation of mycelia and agar, particularly at temperature above 30°*C*. A correlation between colony morphology of tested strains and host trees was revealed. The Ff/8 isolated from *J. regina* form woolly-cottony white to cinnamon-brown colony with dark brown agar, the Ff/11 from *Fagus* tree – cottony, white to creamy-cinnamon colony with bleached, but in some

places yellow-brown agar, Ff/14 and Ff/18 from deciduous trees – cottony, white to cinnamon-brown in the center of colonies with dark brown agar, Ff/16 from *S. alba* – cottony, white colony with unchanged agar and the Ff/26 from *Carpinus* tree – woolly white, in some places creamy to cinnamon colony with unchanged agar (Tabs. 1 and 3). The development of primordia and fruiting bodies was observed after 21 days of growth at the dark in 1 month cultures of Ff/8, Ff/14, Ff/18 and Ff/26 strains on MEA (Fig. 2, a–d).

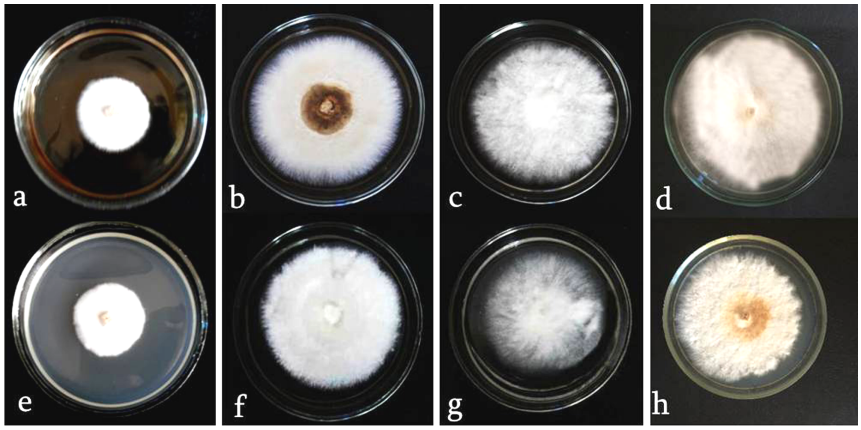

Fig. 1. Colony morphology of *F. fomentarius* on MEA (a–d) and PDA (e–h) on the 6th day of growth at 25°C: Ff/11 (a, e); Ff/18 (b, f); Ff/16 (c, g) and Ff/26 (d, h).

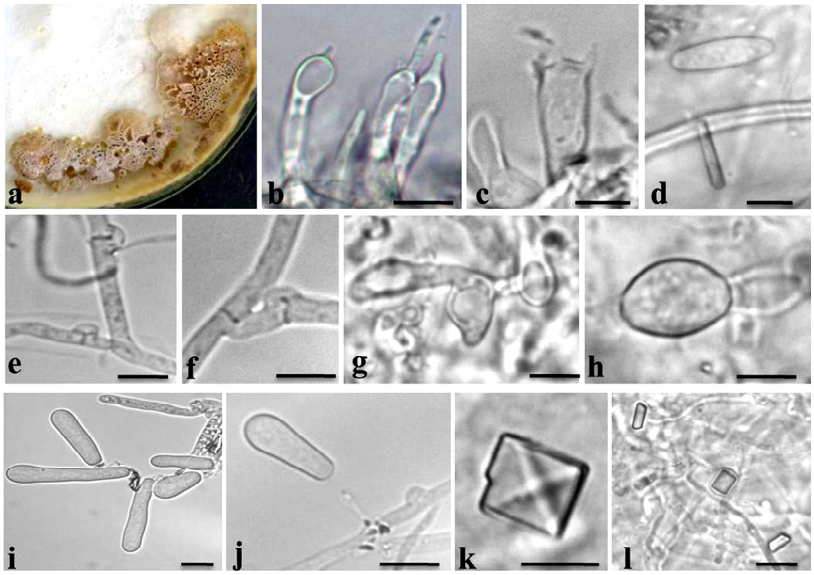

Fig. 2. Development of fruiting bodies in strain Ff/26 of *F. fomentarius* on MEA: hymenium (a), cystidium (b), basidium (c) and basidiospore (d). Hyphal clamps in Ff/14 (e) and Ff/16 (f); chlamydospore-like swellings in Ff/16 (g); chlamydospores in Ff/18 (h); oidia in Ff/18 (i) and Ff/11 (j); crystals in Ff/8 (k) and Ff/16 (l) strains.

The dikaryotic mycelium of *F. fomentarius* possesses single, round-shaped hyphal clamps almost at each septum (Fig. 2, e and f). The presence of arthrospores

(thalloconidia) and chlamydospores on dikaryotic and monokaryotic mycelia of *F. fomentarius* was described [11, 22]. In our collections the apically-swelled chlamydospore-like structures (7.27–10.92×5.53–5.98 *μm*) and intercalary chlamydospores (8.33–14.22×6.59–12.55 *μm*) were observed in Ff/11, Ff/16, Ff/18 and Ff/26 strains (Fig. 2, g and h), while cylindrical arthrospores (oidia) (14.22–27.34×3.63–8.68 *μm*) – in Ff/11, Ff/16 and Ff/18 strains (Fig. 2, i and j). The oidia and chlamydospores have not been observed in Ff/8 and Ff/14. The tetrahedral and cylindrical crystals were abundantly formed in all strains (Fig. 2, k and l).

Growth Characteristics of Mycelia at Different Temperatures. It was reported that temperature range 25–30º*C* is favorable for mycelial growth of *F. fomentarius* [11, 13]. The optimal growth temperature is 25º*C* (3.0–4.4 *mm*/*d* and 2.1–4.2 *mm*/*d* on MEA and PDA, respectively) for Ff/11 and Ff/18 strains, while for Ff/8, Ff/14, Ff18 and Ff/26 it is 30º*C* (4.8–6.0 *mm*/*d* and 4.0–5.3 *mm*/*d*, on MEA and PDA, respectively) (Tab. 2). The Ff/11 and Ff/18 strains do not grow under temperatures above 30º*C*, while others tolerate temperature 35º*C* and above (Tab. 3, Fig. 3). Thus, the southern Armenian strains of *F. fomentarius*, except Ff/11, similar to strains isolated from Italy, prefer optimal growth temperature 30º*C* and above in comparison with northern Austrian strains, which grow better at 25º*C* [13]. Based on obtained data, a correlation between temperature and GR was revealed: a higher temperature was associated with faster growth of strains (Tab. 2).

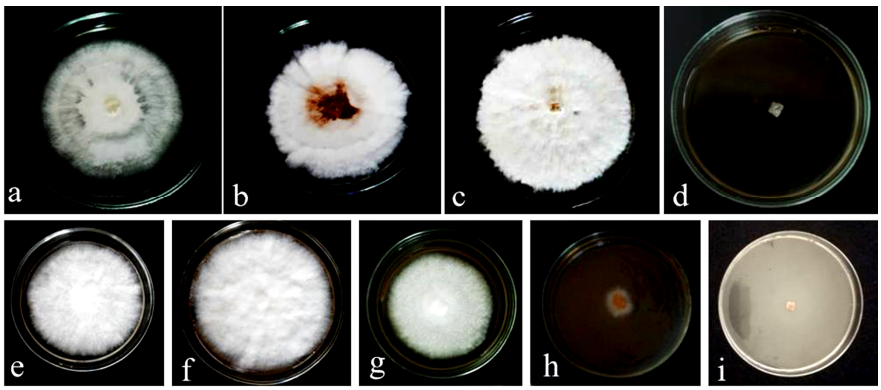

Fig. 3. Colony morphology of *F. fomentarius* strains at different temperature on MEA: Ff/8 (a–d) and Ff/16 (e–i); 25º*C* (a, e); 30º*C* (b, f); 35º*C* (c, g); 38º*C* (d, h) and 40º*C* (i).

Table 2

The GR_{avr} (mm/d) of F. fomentarius strains at different temperatures on different agar media

Strain	25º*C*		30º*C*		35º*C*		38º*C*		40º*C*	
	MEA	PDA	MEA	PDA	MEA	PDA	MEA	PDA	MEA	PDA
Ff/8	4.3±0.42	3.2±0.24	5.5±0.62	4.0±0.40	4.6±0.34	2.7±0.22	GI	GI	NG	NG
Ff/11	3.0±0.26	2.1±0.12	2.8±0.21	2.1±0.19	GI	GI	NG	NG	–	–
Ff/14	4.5±0.13	3.4±0.22	4.8±0.29	3.8±0.22	1.2±0.12	1.8±0.17	NG	NG	–	–
Ff/16	4.5±0.15	4.1±0.32	6.0±0.22	5.3±0.63	4.8±0.46	2.9±0.19	0.9±0.08	0.8±0.05	GI	GI
Ff/18	4.4±0.22	4.2±0.35	3.5±0.19	3.0±0.34	GI	GI	NG	NG	–	–
Ff/26	5.1±0.52	4.5±0.36	5.8±0.56	5.0±0.42	2.4±0.24	1.9±0.15	1.9±0.13	1.5±0.09	GI	GI

Note: MEA – malt-extract-agar; PDA – potato-dextrose agar; GI – growing on inoculum; NG – not growing; (–) – not tested.

Depending on the hardness of wood, the host trees of *F. fomentarius* could be presented in the following order: *J. regina* (very hard wood), *Fagus sp*. (hard wood), *Carpinus sp*. (medium hard wood) and *S. alba* (soft wood). The correlation between hardness of wood substrate, mycelial GR and temperature has not been revealed in the tested collection of *F. fomentarius*. *Fagus*-derived Ff/11 strain grows slower at 30ºC with optimal temperature 25ºC, while strains Ff/8 from *J. regia,* Ff/16 from *S. alba* and Ff /26 from *Carpinus* trees reveal higher growth kinetics and tolerate 35–40ºC temperature range with optimal 30ºC (Tabs. 2 and 3). Meanwhile, a correlation between host wood and pigmentation of colony/agar was found. The mycelia were creamy-cinnamon in Ff/11 and Ff/26 from *Fagus* and *Carpinus* trees and cinnamon-brown in Ff /8 and Ff /18 from *J. regina* and decidious tree, respectively. The agar was unchanged in Ff/16 and Ff/26, bleached with yellow-brown spots in Ff/11 and dark brown in Ff/8, Ff/14 and Ff/18 strains. The pigmentation of mycelium and agar was absent in *S. alba* –derived strain Ff/16 (Tab. 3).

Table 3

The pigmentation, growth, temperature and host tree characteristics of studied collections of F. fomentarius

Strain	Pigmentation of mycelium / agar	Range of GR_{avr} (*mm*/*d*) on MEA / PDA	Temperature optimum / max, ºC	Host tree
Ff/8	white cinnamon-brown / dark brown	5.5 / 4.0	30.0 / 35.0	*Juglans regina*
Ff/11	creamy-cinnamon / bleached, partially yellow-brown	3.0 / 2.1	25.0 / 30.0	*Fagus sp.*
Ff/14	cinnamon brown / dark brown	4.8 / 3.4	25.0 / 35.0	Deciduous tree
Ff/18	cinnamon-brown / dark brown	4.4 / 4.2	30.0 / 30.0	Deciduous tree
Ff/16	white / unchanged	6.0 / 5.3	30.0 / >35.0	*Salix alba*
Ff/26	creamy-cinnamon / unchanged	5.8 / 5.0	30.0 / >35.0	*Carpinus sp.*

Conclusion. The screening of morphological and growth characteristics of Armenian strains of *F. fomentarius* at different temperatures and on different agar media revealed that the optimal growth condition for mycelia is 25–30ºC on MEA. A correlation between GR and temperature was revealed, while correlation between host tree and GR/temperature has not been observed. The cultural characteristics of *F. fomentarius* (colony morphology, GR, hyphal clamps, chlamydospores, oidia, crystals) are taxonomically valuable and will assist in the correct species identification, and control the purity of cultures during their biotechnological cultivation in order to obtain mycelial biomass and bioactive compounds.

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