MYCOBIOLOGY
2022, VOL. 50, NO. 4, 238–243
https://doi.org/10.1080/12298093.2022.2112586

RESEARCH ARTICLE

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Effect of Temperature, pH, and Media on the Mycelial Growth of *Tuber koreanum*

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ABSTRACT

Members of the genus *Tuber* are ectomycorrhizal fungi; this genus includes more than 180 species worldwide. In the present study, the optimal pH, temperature, and medium suitable for the mycelial growth of the Korean truffle, *Tuber koreanum*, were determined. Mycelium of *T. koreanum*, isolated from fruiting bodies collected in Korea, was used to investigate the effects of these environmental factors. The results showed that malt extract agar and potato dextrose agar were the most suitable for the mycelial growth of *T. koreanum* when cultured at a pH of 6.0 at 25 °C for 30 days.

ARTICLE HISTORY
Received 24 May 2022
Revised 21 July 2022
Accepted 9 August 2022

KEYWORDS
Ectomycorrhizal fungi; mycelium; truffle; *Tuber koreanum*

1. Introduction

There are more than 180 fungal species belonging to the genus *Tuber* (Ascomycota) worldwide, and these species form hypogenous fruiting bodies called truffles [1]. They have ectomycorrhizal (ECM) relationships with the roots of host plants, such as oaks or hazels [2,3]. Truffles require specific soil pH, climate, latitude and altitude conditions for optimal growth [4]. The optimal pH level for mycelial growth of truffles differs for each species [5]. In addition, truffles can generally withstand extreme temperatures; however, the optimum growth temperature could vary depending on the species. The optimal temperature for the mycelial growth for most truffles has been reported to be 22–24 °C, and the average annual temperature of the area where the fruiting bodies grow is known to be approximately 20 °C [6]. Furthermore, it is assumed that different species have specific nutritional preferences [7,8]. Therefore, it is essential to study the environmental conditions required by each *Tuber* species for optimal growth. The soil pH level, temperature, and nutrients required for truffle growth in the field are similar to those required for *in vitro* mycelial growth. Therefore, it is possible to understand the environmental conditions for optimum truffle growth through mycelial experiments [4].

Fruiting bodies of the Korean truffle, *Tuber koreanum* Park & Eom, were first collected from the rhizosphere of *Quercus aliena*, in 2020, in Korea [9]. *T. koreanum* is a white truffle with yellowish- to grayish-brown gleba, partially mixed with bright white mycelium, and some parts of the mature ascospores are light brown. In present study, the optimal pH, temperature, and medium suitable for the mycelial growth of *T. koreanum*, were determined.

2. Materials and methods

2.1. Isolation of mycelia

The surface of the fruiting body for *T. koreanum* was sterilized with 70% ethanol (EtOH). The gleba tissue was then cut into small pieces and sterilized with 70% EtOH for 1 min, followed by 30% H_2O_2 for 1 min. The pieces were placed at the center of malt extract agar (MEA) in a dark room at 25 °C to observe mycelium formation. After confirming that the mycelium stretched out from the fruiting body, it was purely isolated through sub-culturing 2–3 times [10].

2.2. Determination of the optimal mycelial growth condition

To determine the optimal pH and temperature for *T. koreanum* mycelium growth, MEA and potato dextrose agar (PDA) media were prepared at four pH conditions (pH 5.0, 6.0, 7.0, and 8.0), which were adjusted using sodium hydroxide and 2-morpholino ethane sulfonic acid, both of which are weak buffers. After autoclaving at 121 °C for 20 min, 250 mL of each medium was poured into a petri dish (90 mm, SPL, Pocheon-si, South Korea). The pieces of mycelium were placed at the center of

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Table 1. Composition of the growth media used in this study.

Growth medium	Composition
Corn meal agar (CMA)	Corn meal (50 g/L), agar (15 g/L)
Malt extract agar (MEA)	Maltose (12.75 g/L), dextrin (2.75 g/L), peptone (0.78 g/L), agar (15 g/L)
Modified Melin-Norkrans agar (MMNA)	Malt extract (15 g/L), peptone (5.82 g/L), agar (15 g/L)
Oatmeal agar (OMA)	Oatmeal (60 g/L), agar (12 g/L)
Potato dextrose agar (PDA)	Glucose (20 g/L), potato starch (4 g/L), agar (15 g/L)
Sabouraud dextrose agar (SDA)	Glucose (40 g/L), peptone (10 g/L), agar (15 g/L)
Yeast malt extract agar (YMA)	Glucose (10 g/L), malt extract (3 g/L), peptone (5 g/L), yeast extract (3 g/L), agar (15 g/L)

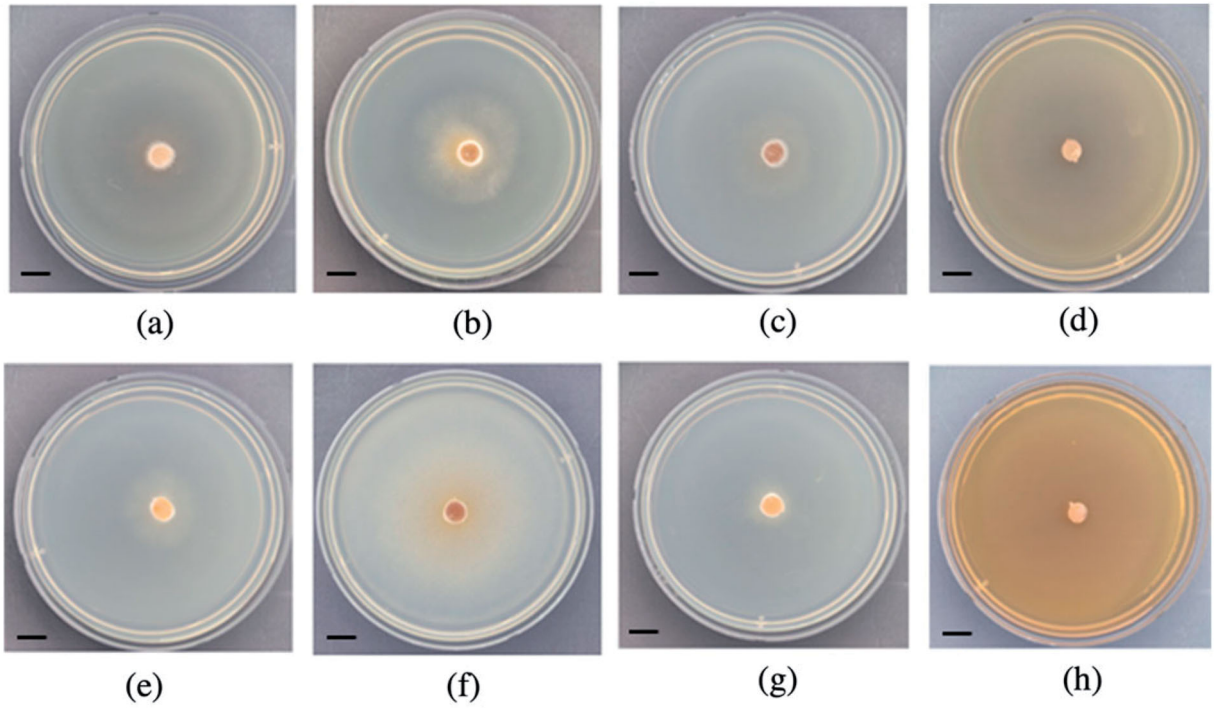

Figure 1. The colonies of *Tuber koreanum* grown under different pH conditions for 30 days. (a, e) pH 5; (b, f) pH 6; (c, g) pH 7; (d, h) pH 8. The growth media used were: PDA (a–d) and MEA (e–h) (scale bar = 1 mm).

MEA and PDA media plates and cultured for 30 days in a dark room at four different temperatures (18 °C, 22 °C, 25 °C, and 28 °C). The diameters of the grown colonies were measured and compared under each pH and temperature condition. An appropriate medium for the growth of *T. koreanum* mycelium was investigated at the optimal culture conditions of pH 6.0 and 25 °C. Seven different growth media were used, namely, corn meal agar (CMA), MEA, modified Melin-Norkrans agar (MMNA), oatmeal agar (OMA), PDA, Sabouraud dextrose agar (SDA), and yeast malt extract agar (YMA), and their compositions are listed in Table 1.

3. Results

3.1. Effects of pH

Differences in the mycelial growth of *T. koreanum*, were observed at different pH conditions (pH 5.0, pH 6.0, pH 7.0, and pH 8.0). The highest mycelial growth occurred at pH 6.0 in both PDA and MEA media after 30 days of culturing at all the temperature conditions (18 °C, 22 °C, 25 °C, and 28 °C) as shown in Figures 1 and 2. Subsequently, significant differences ($p < 0.001$) in mycelial growth at different pH conditions were determined by an independent one-way univariate GLM analysis.

3.2. Effects of temperature

After culturing at four different temperatures (18 °C, 22 °C, 25 °C, and 28 °C), the highest mycelial growth was observed at 25 °C in both PDA and MEA media under all pH conditions (pH 5.0, 6.0, 7.0, and 8.0) as shown in Figures 2 and 3. Significant differences ($p < 0.001$) in mycelial growth at different temperatures were determined by an independent one-way univariate GLM analysis.

3.3. Effects of growth media

Differences in the growth of *T. koreanum* mycelium were observed on different growth media (pH adjusted to 6.0) after 30 days of culturing at 25 °C. Among the seven media tested (CMA, MEA, MMNA, OMA, PDA, SDA, and YMA), MEA and PDA were the most suitable for mycelial growth, while there was no growth on SDA (Figure 4). The

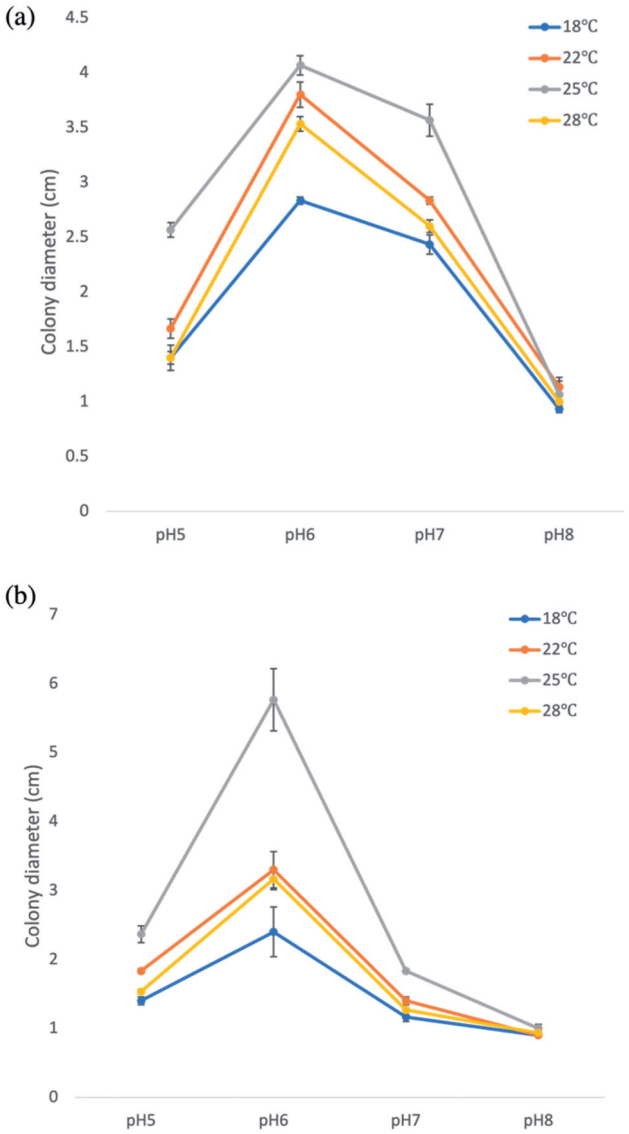

Figure 2. Mycelial growth of *Tuber koreanum* under different pH and temperature conditions. Mean colony diameters (±standard errors) were measured on potato dextrose agar (PDA) (a) and malt extract agar (MEA); (b) after 30 days of culture.

ANOVA analysis revealed significant differences between the growths of the mycelia on different media ($p < 0.001$). Additionally, the mycelium appearance differed in each medium, as shown in Figure 5. On CMA, the mycelia grew around the inoculum at the center of the medium plate; however, filamentous colonies with indistinct outlines due to the low uniform density at the edges were observed. On MEA, MMNA, OMA, and YMA plates, the mycelium appeared as circular colonies growing at a constant rate around the inoculum at the center. On PDA, the mycelium was dense and grew at an irregular rate around the central inoculum, showing growth characteristics that did not form in a circle.

4. Discussion

It is important to understand the environmental conditions preferred by the *Tuber* spp. for their growth [4]. However, studies on the environmental conditions for seedling growth have been considered difficult because they are time-consuming, and other conditions are difficult to control [11]. The soil conditions for mycelium growth and the area where fruiting bodies and ECM are found have been reported to be almost similar [4]. Therefore, by understanding the ideal growth conditions required mycelium, it is possible to determine the preferred environmental growth conditions for *Tuber* spp. [4].

According to previous studies, *Tuber* spp. generally prefer alkaline soil; however, there is a difference in the preferred pH across species [12]. Indeed, the optimum pH for the mycelial growth of *T. borchii* is 6.5, although the fruiting bodies mainly occur in neutral soil [13]. For *T. japonicum*, the optimal pH is 5.0–6.0, and fruiting bodies occur in weakly acidic soil [14]. *T. magnatum* showed optimal mycelial growth at pH 6.0, and the fruiting

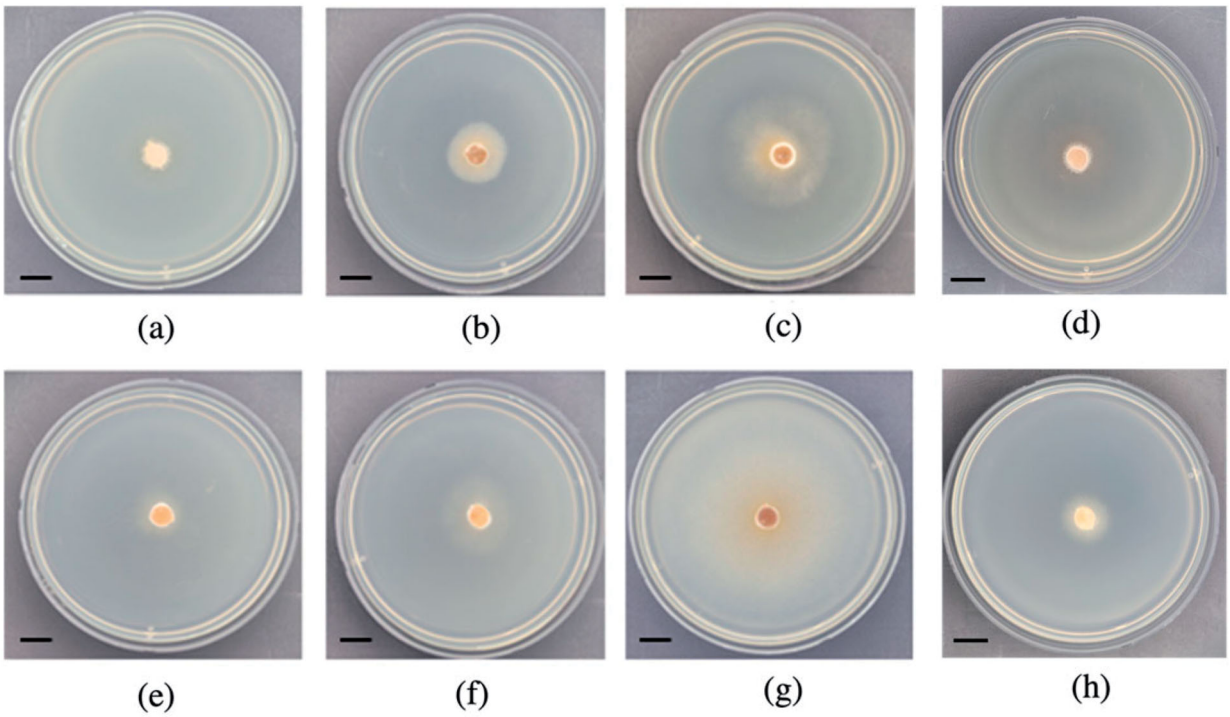

Figure 3. The colonies of *Tuber koreanum* grown under different temperature conditions for 30 days. (a, e) 18 °C; (b, f) 22 °C; (c, g) 25 °C; (d, h) 28 °C. The growth media were: potato dextrose agar (PDA) (a–d) and malt extract agar (MEA) (e–h) (scale bars = 1 mm).

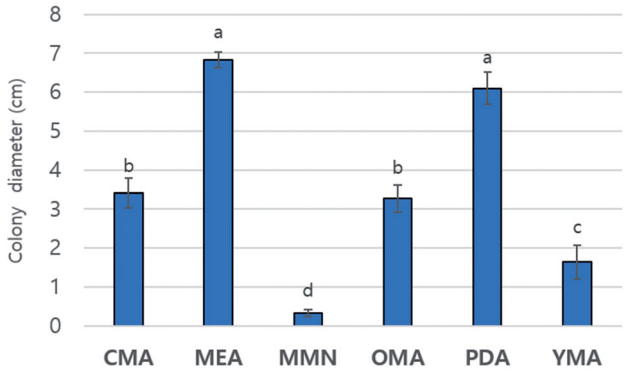

Figure 4. Mean colony diameters (±standard errors) of *Tuber koreanum* on different media after 30 days of culture. Different letters above the bars indicate significant differences at $p < 0.05$ (LSD test). CMA: corn meal agar; MEA: malt extract agar; MMNA: modified Melin-Norkrans agar; OMA: oatmeal agar; PDA: potato dextrose agar; SDA: Sabouraud dextrose agar; YMA: yeast malt extract agar.

bodies occur in soils with similar pH levels [15]. Investigation of the most suitable pH for *T. koreanum* mycelial growth revealed an optimum pH of 6.0.

The optimum temperature for the mycelial growth of *T. koreanum* was observed to be 25 °C. In general, the optimal temperature for mycelial growth differs according to the *Tuber* species [12]. For example, the optimum temperature for the mycelial growth of *T. melanosporum* and *T. magnatum* was 25 °C [16] and 20 °C [17], respectively.

The suitable media for the optimal growth of *T. koreanum* mycelium were found to be MEA and PDA. Previous studies have shown that mycelial growth is stimulated in presence of specific composition of growth medium [18]. For example, the mycelia of *T. borchii* prefer a medium containing glucose and fructose but cannot grow well in those containing sucrose [7]. In contrast, the mycelia of *T. melanosporum* can be grown in a medium containing both sucrose and mannose [8]. In addition, *T. borchii* generally favors glucose; however, mycelial growth is inhibited in a medium with a high glucose concentration [19]. It was confirmed that MEA and PDA were the most optimal medium for growth of *T. koreanum* among the various medium used in the current study. However, the composition and concentration of each medium under specific conditions were not investigated here; therefore, further relevant studies are necessary.

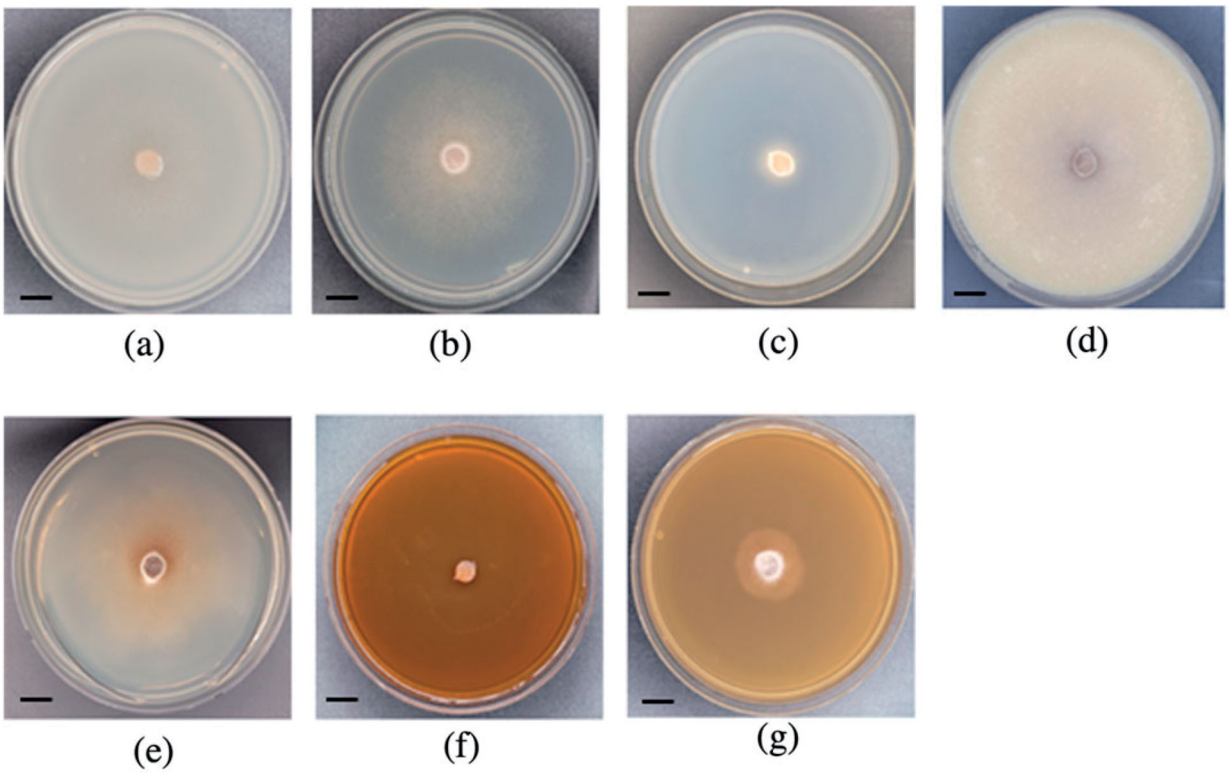

Figure 5. The colonies of *Tuber koreanum* grown on different media for 30 days at pH 6 and 25 °C. (a) Corn meal agar (CMA); (b) malt extract agar (MEA), (c) modified Melin-Norkrans agar (MMNA); (d) oatmeal agar (OMA); (e) potato dextrose agar (PDA); (f) Sabouraud dextrose agar (SDA); (g) yeast malt extract agar (YMA). Scale bar = 1 mm.

In the present study, we investigated the most suitable mycelial growth conditions for cultivating the Korean native truffle, *T. koreanum*. According to the results, MEA and PDA were the most suitable media for the mycelial growth of *T. koreanum* when cultured at a pH of 6.0 and a temperature of 25 °C for 30 days. We confirmed that the mycelial growth of *T. koreanum* required unique conditions compared to those of the other *Tuber* species. Since the preferred conditions, such as temperature, pH, and nutrients, can vary between different *Tuber* species, these factors should be considered in the growth of *Tuber* spp.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (IPET319106052HD050).

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