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Optimal Growth Conditions of Microorganisms Isolated from the Buyeo Royal Tomb No. 1 and Their Effects on Painting Layers

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Abstract

The Buyeo Royal Tomb No. 1 is an ancient tomb built in the late 6th and early 7th century. The four walls of the main room have murals of four guardian deities, and the ceiling has murals of lotus and cloud patterns. This study assessed the optimal growth conditions of two fungal (*Fusarium oxysporum*, *Mortierella* sp.) and four bacterial (*Bacillus cereus*, *Cupriavidus campinensis*, *Streptomyces avidinii*, *Streptomyces cirratus*) strains isolated from the Tomb No. 1, along with their effects on the painting layer. The two fungi showed optimal growth at 20˚C - 30˚C under both nutrient and non-nutrient conditions. These strains did not decompose or discolor the three pigments (cinnabar, hematite, oyster shell white); however, *M.* sp. showed slight decomposition of the media (starch paste, sea weed). The four bacterial strains showed the most active growth at 20˚C - 25˚C under nutrient conditions and did not grow under non-nutrient conditions. These bacteria commonly degraded animal glue and sea weed components. In addition, *S. cirratus* degraded starch. The genus *Streptomyces* discolored the pigment medium to brown and black, suggesting a possible risk of discoloration of the murals. The current environment in Tomb No. 1 was sufficient for microorganism growth, and the presence of strains such as soil bacteria and *actinomycetes* on the mural surface may damage the murals. The findings of this study could be helpful for preserving mural tombs against biological damage caused by microorganisms that are already present or may be present in the tombs in the future. These findings also provide guidelines for comprehensive conservation management.

Keywords

Ancient Mural Tomb, Murals, Biodeterioration, Buyeo Royal Tombs, Growth

Condition, Painting Layer

1. Introduction

The Three Kingdoms period refers to the period when Goguryeo, Baekje, and Silla developed into civilized countries in Manchuria and the Korean Peninsula from the 1st to the 7th century B.C. The Buyeo Royal Tombs, which are the main subjects of this study, were built in the late 6th to early 7th century during the Baekje era. The tomb group is presumed to be the tombs of the kings and the royal family, consisting of a total of 7 tombs (Figure 1(a)). The Buyeo Royal Tombs were designated as Historic Site No. 14 and were included in the 'Baekje World Historic Area' along with other cultural heritage sites such as the Gongju Royal Tombs. "The Buyeo Royal Tombs" were registered as a UNESCO World

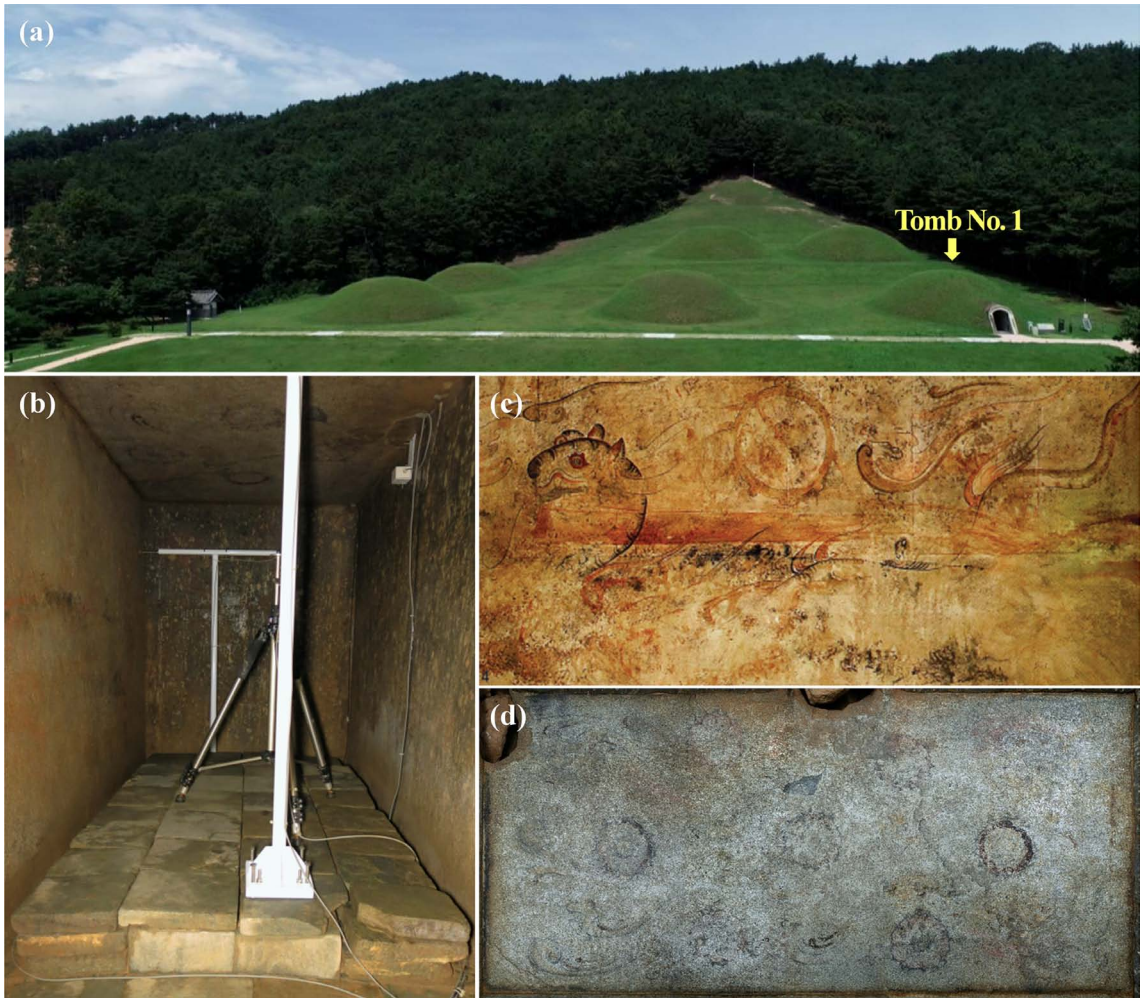


Figure 1. Details of the Royal Tomb No. 1. (a) Aerial photograph of the Tomb No. 1, (b) The inside of the main room in Tomb No. 1, (c) A replica of murals painted on the west wall of main room, (d) Murals (lotus and cloud patterns etc.) on the ceiling wall of main room. (photo source: Baekje World Heritage Center, Buyeo National Museum, Kunkuk University).

Heritage Site in 2015.

The Buyeo Royal Tomb No. 1 (Tomb No. 1) is the only tomb of the seven tombs with murals (**Figure 1(a)**). The four walls of the main chamber are painted with depictions of the four guardian deities, and lotus and cloud patterns are painted on the ceiling (**Figure 1(c)** & **Figure 1(d)**). The murals were produced using the "Jobyeokji Technique" of painting murals directly on the flat surface of the stone slab without creating a base layer [1]. The murals have become an important material for studying the art of Baekje painting.

The exterior of Tomb No. 1 is a circular mound tomb made of soil, and the interior is a corridor-style stone chamber tomb with a corridor and the main chamber (**Figure 1(a)** & **Figure 1(b)**). The main chamber was constructed using three large stone slabs to form each wall to the east, west, and north with a covering stone on the top of the walls (**Figure 1(b)**). Mural tombs with a similar structure consisted of a narrow and closed space and have been preserved unaffected by the external environment for a long time. However, artificial opening activities such as robbery and excavation have altered the unique environment of the tombs, accelerating damage to the murals [2] [3].

In tomb murals, various factors such as temperature, light, and airflow cause physical, chemical, and biological damage. In particular, during biological damage, microorganisms can grow under environmental influences such as temperature, humidity, and light. The mean annual temperature and relative humidity of mural tombs in the Republic of Korea were 16.2˚C (maximum temperature: 19˚C - 21˚C) and 99% - 100%, respectively [1] [4] [5]. Similarly, hypogeal cultural heritage sites in other countries generally have a temperature of 12˚C - 22˚C and relative humidity greater than 90% [6] [7] [8] [9]. Psychrophilic and mesophilic microorganisms can grow in mural tomb environments. *Aspergillus*, *Cladosporium*, *Fusarium*, and *Pseudomonas*, which have been isolated from the Tomb No. 1, have also been found in the Gongju Royal Tomb No. 6 in the Republic of Korea and the Takamatsuzuka Tomb in Japan [10] [11]. In addition, studies have also reported that microorganisms grow by utilizing the materials (medium, organic matter, etc.) for the murals as a nutrient source [3] [12], and their growth may be enhanced by the material (methylcellulose, ethanol, isopropanol, etc.) used for the preservation of the murals [13] [14] [15] [16].

The following types of damage may appear on the mural tombs according to the generation and growth of microorganisms. 1) Penetration of the mycelial growth of mold and *actinomyces* into the murals causing physical damage [17]. When the mycelium penetrates into the colored layers, the layers lose their cohesiveness, become powdered, and are eventually peeled off and exfoliated [17] [18]. 2) Murals can be damaged by secondary metabolites from microorganisms [19], and protective pigments are sometimes secreted under poor environmental conditions [20]. 3) Murals can be damaged by enzymes of starch, protein, and fat from microorganisms, and these enzymes may decompose the medium [21].

Studies must investigate the physiological characteristics, causes, and risks of damage caused by microorganisms isolated from mural tombs. In other coun-

tries, studies have actively evaluated the relationship between the growth of organisms and factors such as temperature, light, and carbon dioxide [22] [23] [24] [25]. In contrast, studies in the Republic of Korea have only investigated the biochemical characteristics of fungi isolated from the Gongju Royal Tomb No. 6 [26]. Although these studies examined the optimal culture conditions and enzyme activity of fungi, further studies have not been conducted. Additional studies must be conducted to assess the relationship between various elements of mural tombs and the growth of microorganisms.

Therefore, this study aimed to assess the optimal conditions for the growth of microorganisms, and the effects of the microorganisms on painting layers in the Buyeo Royal Tomb No. 1 in the Republic of Korea. The optimal growth temperature and nutrient source of microorganisms were selected by recreating the environment of the Buyeo Royal Tomb No. 1. To understand the effects of microorganisms on painting layers, decomposition and discoloration properties of the microorganisms were assessed in pigments and mediums of the murals.

2. Materials and Methods

2.1. Selection of Test Strains

The test strains were selected by applying the following four criteria based on the results of previous studies on the distribution of microorganisms in the Buyeo Royal Tomb No. 1: 1) Isolation for each investigation; 2) High microbial distribution ratio (%); 3) Distribution in domestic and foreign mural tombs; 4) One strain selected from each phylum unit if many identical species were observed. As a result, two strains of fungi (*Fusarium oxysporum*, *Mortierella* sp.) and four strains of bacteria (*Bacillus cereus*, *Cupriavidus campinensis*, *Streptomyces avidinii*, *Streptomyces cirratus*) were selected (Table 1). These strains were received from the Institute of Preventive Conservation for Cultural Property at the Korea National University of Cultural Heritage.

Table 1. List of test strains.

No.	Sample Name	Description	Accession No.	Pct. (%)
1	F1	*Fusarium oxysporum*	XR_001936467.1	99
2	F2	*Mortierella* sp.	KY773292.1	99
3	B1	*Bacillus cereus*	NR_114582.1	99
4	B2	*Cupriaviuds campinensis*	NR_025137.1	99
5	B3	*Streptomyces avidinii*	NR_041132.1	99
6	B4	*Streptomyces cirratus*	NR_043356.1	99

2.2. Optimal Growth Conditions for Microorganisms

2.2.1. Nutrient and Temperature

The Tomb No. 1 is currently closed to the public to preserve its original form. Access is restricted for all except for those entering for management and investigation pur-

poses. There is no artificial lighting inside the Tomb No. 1, and the relative humidity is maintained at 99% - 100%. Therefore, in this study, nutrients and temperature were selected as conditions that could affect the growth of microorganisms.

The nutrient and non-nutrient media used for the fungi were potato dextrose agar (PDA, Difco, USA) and agar (Agar, Difco, USA), respectively. The fungi were sampled from the tip of the pre-cultured mycelium and cultured in the center of the medium at a temperature of 10˚C, 15˚C, 20˚C, 25˚C, and 30˚C for seven days. The diameter (mm) of mycelial growth was measured at intervals of 24 h using vernier calipers.

The nutrient and non-nutrient media for the bacteria were nutrient broth (NB, Difco, USA) and distilled water, respectively. Pre-cultured bacteria were inoculated to have the initial medium at a concentration of 0.5 McFarland (OD 0.08 - 0.10, measured using spectrophotometer of 660 nm). Next, the bacteria were cultured under the same temperature conditions as the fungus (10˚C, 15˚C, 20˚C, 25˚C, and 30˚C) in a shaking incubator (150 rpm, 72 h), and the culture medium was recovered at 6, 12, 18, 24, 36, 48, 60, and 72 h. To measure the turbidity of the culture medium, absorbance was assessed at 660 nm using a spectrophotometer (UVmini-1240, SHIMADZU, JPN).

2.3. Growth Relationship with Painting Layers

2.3.1. Pigment Decomposition and Discoloration

Red, yellow, black, white, and brown pigments were used for the murals of the Tomb No. 1. The presumed pigments for red, white, black, and brown colors were Cinnabar (HgS), Oyster shell white ($CaCO_3$), Carbon black (C), and Hematite (Fe_2O_3), respectively.

In this study, Cinnabar (HgS, Co. Gail traditional pigment, Gyeonggi-do, Korea), Hematite (Fe_2O_3, Co. Gail traditional pigment, Gyeonggi-do, Korea), and Oyster shell white ($CaCO_3$, Co. Gail traditional pigment, Gyeonggi-do, Korea) were selected as the testing materials. A pigment medium (0.5% pigment per 1 L of distilled water) was prepared by mixing the pigments with the microbial medium (fungi: PDA medium, bacteria: TSA medium). The fungi were inoculated with a fungal block (5 mm in diameter) in the center of the pigment medium, and the bacteria were smeared in zigzag. The control strain was *Aspergillus niger* [25], which was cultured in an incubator for seven days. Pigment decomposition and discoloration around the microorganisms after culture were evaluated.

2.3.2. Medium Decomposition Evaluation

No previous study has identified the medium used in the murals of the Tomb No. 1. Therefore, in this study, starch paste, animal glue, and sea weed were selected among media that were presumed to have been used at the time of mural production. The culture medium was prepared with each medium as the main component, and medium decomposition was assessed by inoculating microorganisms.

1) Starch paste decomposition

Starch paste decomposition was evaluated by preparing a medium containing starch, which is the main component of wheat starch paste (1% starch from wheat, 3.9% PDA per 1 L of distilled water). Fungi were inoculated in the center of the medium with a block made of cork borer (diameter 4 mm), and bacteria were smeared in a straight line after submerging the spreader in the bacteria suspension. The strains were then incubated for two to seven days at 30˚C in an incubator and stained with Lugol's solution (62650, Sigma-Aldrich, USA) for evaluation of starch decomposition.

2) Animal glue decomposition

Animal glue decomposition was evaluated by preparing a medium containing gelatin, which is the main component of cow glue (1.5% gelatine, 0.4% peptone, 0.1% yeast extract, 1.5% agar per 1L of distilled water). The test strains were inoculated and plated using the same method as above and reacted by dropping a mixed solution of 15% $HgCl_2$ and 20% HCl. The reaction solution was removed using distilled water, and the opaque ring which formed around the colonies was assessed to evaluate animal glue decomposition.

3) Sea weed decomposition

Sea weed decomposition was evaluated by preparing a medium containing marine agar (MA 2216, Difco, USA), which is the main component of red algae. The test strains were inoculated and plated using the same method as above and cultured for two to seven days at 30˚C in an incubator. Sea weed decomposition was evaluated by assessing dentation and medium liquefaction around the microbial colony and the resolution displayed after staining with Lugol's solution.

3. Results

3.1. Effect of Nutrients and Temperature

3.1.1. F1 (*Fusarium oxysporum*)

Under nutrient conditions, F1 mycelial growth rate increased with temperature. The mycelial growth length after seven days of culture at 10˚C, 15˚C, 20˚C, 25˚C, and 30˚C was 20.0 ± 0.0 mm, 33.3 ± 0.8 mm, 48.0 ± 0.5 mm, 61.7 ± 2.8 mm, and 66.7 ± 1.3 mm, respectively. Under non-nutrient conditions, F1 showed the highest growth rate at 25˚C with a mycelial growth length of 72.8 ± 1.0 mm after seven days of culture (Table 2).

Table 2. Mycelial growth length of *F. oxysporum* in both nutrient and non-nutrient medium under various temperature conditions.

Nutrient condition	Culture period	Temperature conditions				
		10˚C	15˚C	20˚C	25˚C	30˚C
Nutrient -PDA	7 days	20.0 ± 0.0	33.3 ± 0.8	48.0 ± 0.5	61.7 ± 2.8	66.7 ± 1.3
Non-nutrient -Agar	7 days	20.0 ± 2.0	34.0 ± 0.5	53.8 ± 0.3	72.8 ± 1.0	61.0 ± 1.0

3.1.2. F2 (*Mortierella* sp.)

M. sp. showed maximum growth under nutrient conditions at 20˚C - 30˚C. Mycelial growth length on the 7th day of culture at 10˚C, 15˚C, 20˚C, 25˚C, and 30˚C was 19.0 ± 0.8 mm, 29.0 ± 0.6 mm, 39.0 ± 0.5 mm, 42.0 ± 1.8 mm, and 37.0 ± 1.5 mm, respectively. Under non-nutrient conditions, microscopic observations showed *M.* sp. growing as a mycelium without forming spores (data not shown). Thus, *M.* sp. showed a higher growth rate under non-nutrient conditions than under nutrient conditions. In particular, under non-nutrient conditions, the maximum growth was observed at 20˚C - 30˚C (Table 3).

Table 3. Mycelial growth length of *Mortierella* sp. in both nutrient and non-nutrient medium under various temperature conditions.

Nutrient condition	Culture period	Temperature conditions				
		10˚C	15˚C	20˚C	25˚C	30˚C
Nutrient -PDA	7 days	19.0 ± 0.8	29.0 ± 0.6	39.0 ± 0.5	42.0 ± 1.8	37.0 ± 1.5
Non-nutrient -Agar	7 days	28.0 ± 1.4	44.0 ± 3.4	62.0 ± 0.6	62.0 ± 2.0	62.0 ± 0.5

3.1.3. B1 (*Bacillus cereus*)

B1 showed the highest growth under nutrient conditions at 25˚C. At 15˚C and 20˚C, the growth rate was similar according to the culture time. The growth rate of B1 was the greatest between 18 and 36 h of culture. The initial absorbance of B1 under non-nutrient conditions at every temperature was 0.08 - 0.10 and decreased with culture time (Figure 2).

3.1.4. B2 (*Cupriavidus campinensis*)

The B2 growth rate was the highest at 25˚C and 30˚C. The growth rate was the fastest between zero and six hours of culture, and a slow growth curve was

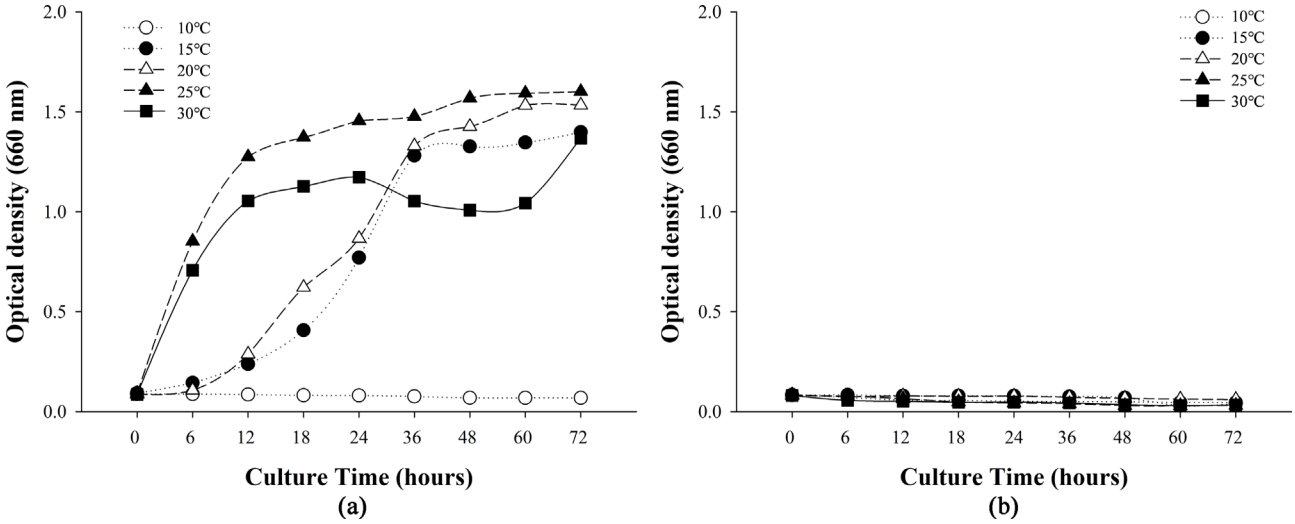


Figure 2. Growth curve of *B. cereus*. (a) Nutrient and temperature conditions; (b) Non-nutrient and temperature conditions.

observed after 12 h of culture. At 20˚C, the growth rate was the highest between six and 18 h of culture. The absorbance was between 1.27 and 1.40 after 18 h of culture. At 15˚C, B2 showed a fast growth rate between 24 and 36 h of culture; however, absorbance was constant ranging between 1.25 and 1.36 after 36 h of culture. Under non-nutrient conditions, absorbance was 0.08 - 0.17 during the culture period and gradually decreased with culture time (Figure 3).

3.1.5. B3 (*Streptomyces avidinii*)

Under nutrient conditions, in B3, the exponential growth rate increased as the temperature increased (10˚C: 60 - 72 h, 15˚C: 24 - 48 h, 20˚C: 12 - 24 h, 25˚C: 6 - 12 h, 30˚C: 6 - 12 h). In particular, B3 showed a fast initial growth rate at 25˚C - 30˚C. The exponential growth phase was between 6 and 12 h of incubation. Afterwards, the growth curve plateaued. Under non-nutrient conditions, B3 absorbance was similar to or decreased than the initial absorbance at all temperatures (Figure 4).

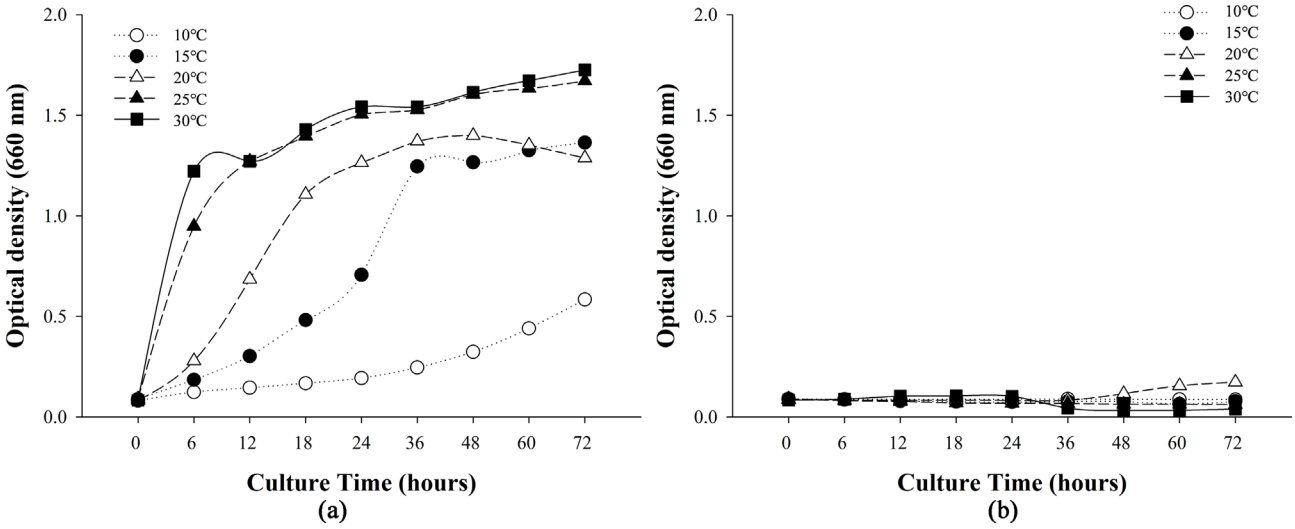


Figure 3. Growth curve of *C. campinensis*. (a) Nutrient and temperature conditions; (b) Non-nutrient and temperature conditions.

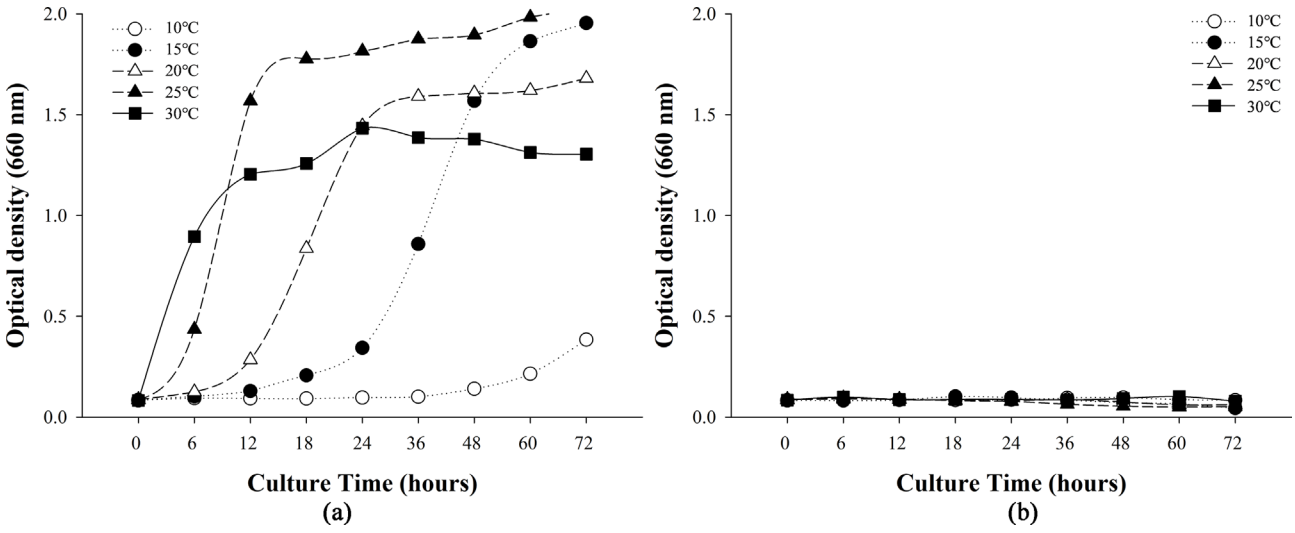


Figure 4. Growth curve of *S. avidinii*. (a) Nutrient and temperature conditions; (b) Non-nutrient and temperature conditions.

3.1.6. B4 (*Streptomyces cirratus*)

Under nutrient conditions, B4 grew rapidly within 12 h of culture at 25˚C - 30˚C; however, the growth curve plateaued after 12 h. At 15˚C and 20˚C, exponential growth of B4 was observed at 24 - 36 h and 6 - 18 of culture, respectively. At 10˚C, absorbance increased gradually with culture time; however, the absorbance was the lowest among all temperature conditions. Under non-nutrient conditions, B4 absorbance was similar to or decreased than the initial absorbance at all temperatures (Figure 5).

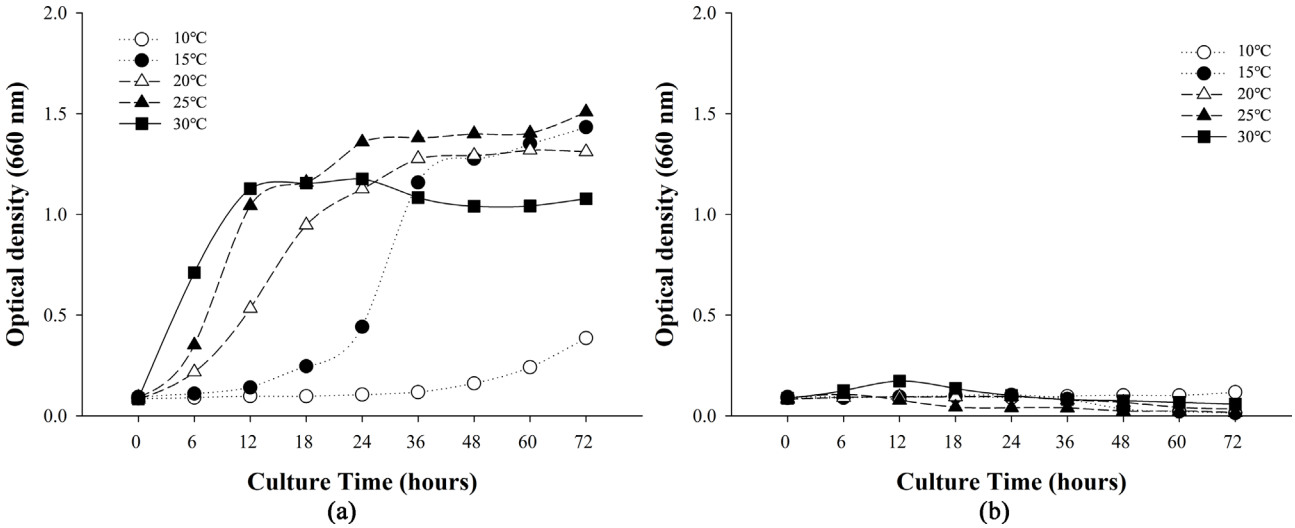


Figure 5. Growth curve of *S. cirratus*. (a) Nutrient and temperature conditions; (b) Non-nutrient and temperature conditions.

3.2. Pigment Decomposition and Discoloration

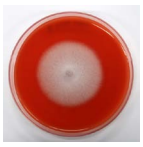
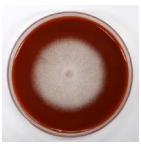
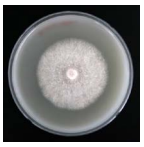
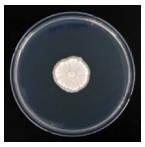
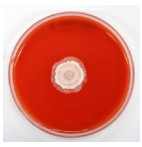
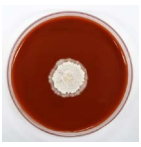
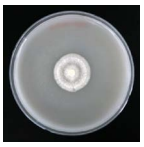
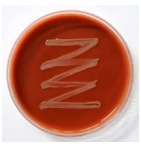
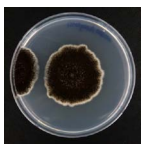
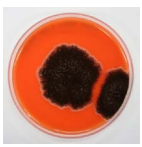
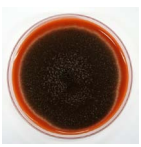
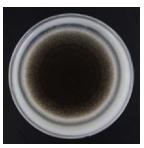
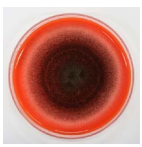
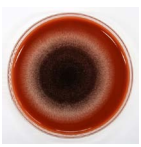
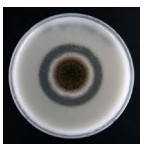
Transparent decompositions rings were not observed around molds F1 and F2 inoculated on cinnabar, hematite, and oyster shell white medium. In addition, pigment discoloration was not observed in all pigment media compared to that in the uninoculated medium. Although no decomposition ring was observed in the pigment media inoculated with four bacterial strains, the medium were discolored to brown around some strains. In particular, brown and black pigmentations were observed around B3 and B4 strain colonies on all media (Table 4).

Transparent decomposition rings were observed around the control strain inoculated on hematite medium. In contrast, no decomposition ring was observed on cinnabar and oyster shell white medium. Compared to the uninoculated medium, no discoloration was observed in all pigment media (Table 4).

3.3. Medium Decomposition

F1 did not decompose the starch, gelatin, and agar medium. In contrast, F2 decomposed starch and agar medium by 0.5 mm and 1.0 mm, respectively. No decomposition was observed on gelatin medium. Among the test bacteria strains, B4 decomposed the media the most: 4.7 ± 0.3 mm on starch medium, 2.3 ± 0.3 mm on gelatin medium, and 12.8 ± 0.4 mm on agar medium. B3, which belonged to the same genus as B4, decomposed both gelatin and agar medium but

Table 4. Results of the pigment degradability and discoloration of the test strains and the control strain.

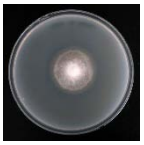
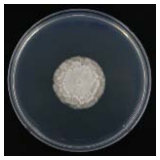
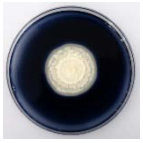
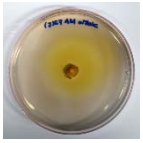
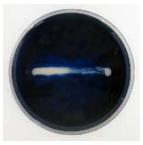
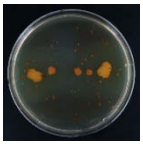
Sample Name	Control	Type of pigment medium		
		Cinnabar	Hematite	Oyster shell white
F1 (*F. oxysporum*)				
F2 (*M.* sp.)				
B1 (*B. cereus*)				
B2 (*C. campinensis*)				
B3 (*S. avidinii*)				
B4 (*S. cirratus*)				
Control-PDA				
Control-TSA				

not starch medium. B1 and B2 showed high medium decomposition of 15.8 mm on agar medium and moderate medium decomposition of 3.5 mm on gelatin medium (Table 5).

4. Discussion

Based on the distribution of microorganisms in the Tomb No. 1, *F. oxysporum*, *M.* sp., *B. cereus*, *C. campinensis*, *S. avidinii*, and *S. cirratus* were selected, and the growth conditions, as well as the effects of these strains on painting layers, were investigated.

Table 5. Results of the medium degradability assessment of the test strains.

Sample Name	Main Component of Medium			
	Control	Starch	Gelatin	Agar
F1 (*F. oxysporum*)				
		-	-	-
F2 (*M.* sp.)				
		0.5 ± 0.0 mm	-	1.0 ± 0.0 mm
B1 (*B. cereus*)				
		-	3.4 ± 0.2 mm	15.7 ± 0.3 mm
B2 (*C. campinensis*)				
		-	3.5 ± 0.5 mm	15.8 ± 0.0 mm
B3 (*S. avidinii*)				
		-	2.7 ± 0.3 mm	7.1 ± 1.0 mm
B4 (*S. cirratus*)				
		4.7 ± 0.3 mm	2.3 ± 0.3 mm	12.8 ± 0.4 mm

4.1. Growth Relationship of Microorganisms with Nutrient Sources and Temperature Conditions

Hypogeal cultural heritage is generally characterized by environmental conditions such as limited air circulation throughout the year, low-temperature changes, and high humidity [6] [27]. Appropriate conditions, including temperature, humidity, and nutrient source, are needed for microbial growth [28] [29] [30]. The environment in Tomb No. 1 was suitable for microbial growth with a

temperature of 14.0˚C - 19.1˚C (based on 2021 data), high relative humidity (~99%, based on 2021 data), no natural light, and preserved nutrient sources with soil and mural materials.

The fungus *F. oxysporum* showed the same life cycle as Ascomycota under both nutrient and non-nutrient conditions. In detail, under nutrient conditions, active growth was observed with increasing temperature. Under non-nutrient conditions, optimal growth was observed at 25˚C. This was consistent with the findings of [31] that *F. oxysporum* can grow at 20˚C - 30˚C. On the other hand, another fungus belonging to the *M.* sp. showed different growth patterns under nutrient and non-nutrient conditions. Under nutrient conditions, optimal growth was observed at 25˚C. In contrast, under non-nutrient conditions, spores that are usually observed in the Mucoromycota life cycle were not formed, mycelial growth was observed instead. The mycelium had a maximum growth rate at 20˚C - 30˚C, which was in agreement with previous findings that *M.* sp. is optimally cultured at 20˚C - 25˚C [32].

Bacterial strains *B. cereus*, *C. campinensis*, *S. avidinii*, and *S. cirratus* showed the most active growth and fast initial growth at 20˚C - 25˚C under nutrient conditions. At 15˚C - 20˚C, all bacterial strains grew to the maximum within 72 h despite showing different initial growth rates. At 10˚C, although the growth of all bacteria was the slowest, all bacteria showed gradual growth. Under non-nutrient conditions, absorbance decreased with culture time at all temperatures, suggesting that the bacteria did not grow.

The strains, excluding *F. oxysporum*, did not grow under non-nutrient conditions nor follow the general rules of microbial growth. However, the tomb offers ideal nutrient sources for the microorganisms used in this study and other sources of nutrients such as soil, pigments, medium, and other contaminants. Hence, if an environment with optimal growth conditions is created, there is potential for microbial growth. In addition, the average temperature of the Buyeo city, where Tomb No. 1 is located, has increased by 0.7˚C compared to 1990 [33], and the actual annual average temperature has been increasing since 2020 (2018: 15.8˚C, 2019: 15.4˚C, 2020: 16.0˚C, and 2021: 16.4˚C). As Republic of Korea continues to experience a hot and humid climate due to climate changes, the actual temperature is expected to keep gradually increasing. Temperature rise, even as small as 2˚C - 3˚C, can increase metabolic activity and growth of microorganisms, altering the species composition [34]. Since the temperature was the most variable environmental factor in Tomb No. 1, regular inspections are required. In addition, a periodical assessment of microorganism distribution should be conducted to ascertain the correlation between temperature and microbial communities in Tomb No. 1.

4.2. Effects of Microorganisms on Painting Layers

To evaluate the effects of the microorganisms on the painting layers, pigments and mediums that were thought to have been used for murals in the Tomb No. 1 were selected. Pigment and medium decomposition and discoloration caused by

the test strains were assessed. *F. oxysporum* and *M.* sp. did not decompose or discolor cinnar, hematite, and oyster shell white medium. In addition, the two strains did not decompose or showed little decomposition of starch, gelatin, and agar medium. These findings suggested that the two strains are likely to have low effects on the painting layers; however, care must be exercised as mold on murals damage the aesthetics [35] [36] [37].

B. cereus, C. campinensis, S. avidinii, and S. cirratus showed high decomposition of gelatin and animal glue, suggesting that they may decompose glue and sea weed. In particular, *S. cirratus* showed the greatest decomposition of medium components. This was consistent with the findings of [21] and [38] that the genus *Streptomyces* can degrade gum, egg yolk, and beeswax. Although no study has evaluated mediums used for murals in the Tomb No. 1, our findings showed that soil microorganisms and actinomycetes isolated from the Tomb No. 1 can decompose the medium components of mural painting layers.

In addition, the genus *Streptomyces* contains various metabolites, including oxalic acid, citric acid, and carotenoids, which may discolor the pigments [8] [17]. Herein, *S. avidinii* and *S. cirratus* isolated from the Tomb No. 1 also discolored the pigment medium to brown and black. Further studies must be conducted to analyze the metabolites of the two strains and the mechanism of pigment discoloration. In other studies on the murals of cultural heritage sites worldwide, *Streptomyces* have been reported to generate red and brown spots and cause biological damage [20] [39] [40] [41]. As *Streptomyces* are the dominant strains on the murals of the Tomb No. 1, the growth of these strains on the murals represents a high risk of damaging the murals.

5. Conclusion

This study analyzed the nutrient sources and temperature conditions that could affect the growth of microorganisms in the Buyeo Royal Tomb No. 1 as well as the effects of the microorganisms on the medium and pigments of the murals. The ancient tomb had sufficient conditions for the occurrence and growth of microorganisms, and the optimal growth temperature for microorganisms was 20˚C - 30˚C. In addition, the growth of strains such as soil bacteria and actinomycetes on the mural surface represents a high risk of biological damage, including discoloration of the pigments and decomposition of the medium in the painting layers. The results of this study may be helpful to preserve mural tombs against biological damage caused by microorganisms that are already present or may be present in the near future. The preservation of the mural tombs painted directly on the stone surface is expected to be possible. These findings also provide guidelines for comprehensive conservation management.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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