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Research Article

Fungal Distribution of the Janggyeong Panjeon, the Depositories for the *Tripitaka* Koreana Woodblocks in the Haeinsa Temple

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ABSTRACT Many investigations have been conducted on the biological damage and environmental conditions necessary to preserve the Janggyeong Panjeon and Daejanggyeongpan (woodblocks). We performed a survey on the concentration and diversity of airborne fungi in the Janggyeong Panjeon and compared them with the results of a survey from 2012. The temperature of the Beopbojeon building was slightly lower, while the relative humidity was higher than those found at the Sudarajang building. The concentration of airborne fungi in the Beopbojeon was 1.44-fold that of the Sudarajang. It was confirmed that the concentration and diversity of airborne fungi in the Janggyeong Panjeon differed depending on the sampling site. In total, 23 fungal genera were identified from the air samples, and 11 fungal and 1 bacterial genera were identified from the surface of the woodblocks. Among these, only five types of fungi were commonly distributed in the indoor air and surface of the Daejanggyeongpan; however, 58.3% of the fungi identified on the surface of the woodblocks were not observed in the in the air samples. The surface-dwelling fungi may accumulate dust to form microbial communities over time.

Key Words Janggyeong panjeon, Daejanggyeongpan, Airborne fungi, Surface-dwelling fungi

1. INTRODUCTION

The Janggyeong Panjeon is the depository that preserves the *Tripitaka* Koreana (Palman Daejanggyeong), which is a collection of 81,350 wooden carved blocks from the 13th century. The Janggyeong Panjeon is located on Mount Gaya, and is placed southwest with the mountain behind it to block humid southeast winds. The layout and structure of the building were meant to be able to preserve the *Tripitaka* Koreana. At the same time, large windows were created to secure abundant sunlight throughout the year; the lower windows were made 3.13 times larger than the top window, and the rear windows 1.45 times larger than the bottom windows (Temple Haeinsa, 2002). The size of the windows were designed to provide natural ventilation allowing the air entering the room to circulate. The floor is also equipped with a natural control device that maintains suitable humidity and temperature in the building by using charcoal, granite, and reinforcement to prevent insects from sucking moisture when drying (Park, 1999). The temple was designed to adapt to climatic conditions, thus preserving the woodblocks from rodent and insect infestation for some 500 years (UNESCO, 1992).

It was designed to prevent biological damage, but over time, various pollutants have accumulated inside the Janggyeong Panjeon causing degradation, especially due to the influence of fungi (Hong *et al.*, 2011). It should be noted that the cultural heritages are susceptible to biological damage caused by insects and microorganisms due to their nature, and once the deterioration begins, the process may be irreversible (Han, 2005). Accordingly, various studies are being conducted on the Janggyeong Panjeon, investigating the ambient airflow and ventilation volume (Hur *et al.*, 1998, Hur *et al.*, 2007; Jo *et al.*, 2017; Lim *et al.*, 2007), the biodeterioration of the square post and woodblocks (Kim *et al.*, 2007a, 2007b; Lee *et al.*, 2018), and the concentration of airborne fungi. In addition, the damage caused by termites, a representative perpetrator of wooden structures, has been diagnosed to design control measures (Jeong *et al.*, 2002; Natural Research Institute of Cultural Heritage, 2014; Hong *et al.*, 2013).

The research on the structure and environment of the Janggyeong Panjeon must therefore be long-term and periodic. In this study, we compare the results on the concentration of indoor airborne fungi in the Janggyeong Panjeon of June 2012 and July 2021, and investigate the association with the fungi collected and cultured from the surface of the Daejanggyeongpan.

2. MATERIALS AND METHODS

2.1. Measurement points

The Janggyeong Panjeon depositories comprise two long and two smaller buildings, which are arranged in a rectangle around a courtyard. The long buildings are the Beopbojeon and Sudarajang, located in the northeast and south to it, respectively. The Beopbojeon has a Buddhist hall in the center and it is arranged as a single space, whereas the Sudarajang is divided into east and west with a passage in the center. Airborne fungi were collected at 10 points in the Beopbojeon and 4 points in the Sudarajang, as shown in Figure. 1.

2.2. Temperature and relative humidity

The temperature and relative humidity (RH) were measured at the point where the fungi were sampled (174 H-2, Testo, Germany). The device automatically measures every 2 s, and the temperature and RH were recorded simultaneously with the microbial collection between 14:00 and 16:00.

2.3. Airborne fungi

The microbial investigation in Janggyeong Panjeon was conducted on July 23, 2021. This survey was the first survey since June 2012, and followed the same approach as that of the previous survey.

In total, 100 L of air was passed through a potato dextrose agar (PDA) (Difco, USA) medium using an air sampler (MairT, Millipore, USA) on a table 70 cm from ground level; samples were collected in triplicate. The collection of

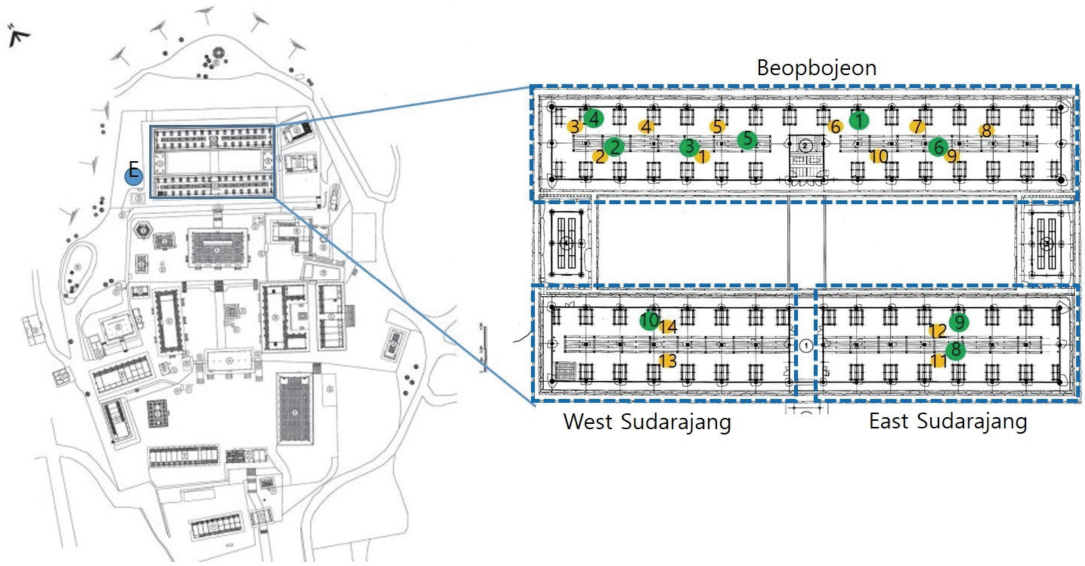

Figure 1. Measurement points for the collection of fungi (orange circles: airborne fungi, green circles: the surface of woodblocks, E: outdoor).

airborne fungi started at Beopbojeon and proceeded to west and east Sudarajang. The collected fungi were cultured for 3 days at 28℃, and the number of colonies per unit volume (CFU/m^3) in the air was calculated as. CFU/m^3 = CFU/ suction flow (L) x 1,000 (L/m^3).

2.4. Collection of fungi from the Daejanggyeongpan

Fungi on the surface of the Daejanggyeongpan were collected using cotton swabs, at various locations as shown in Figure 1.

2.5. Microbial classification and identification

A single colony was isolated by observing the surface shape, size, and color of the colonies on the medium. At this time, colonies presumed to be fungi were cultured in a PDA medium, and those presumed to be bacterial were cultured in NB agar medium at 28℃ for 3 days to 2～3 weeks. Secondarily, the BlastN Search program, a database of the National Center for Biotechnology Information (NCBI), was used to identify the sequencing of the ITS region or 26s rRNA and 16s rRNA region for fungi and bacteria, respectively.

3. RESULTS AND DISCUSSION

3.1. Temperature and relative humidity

The Janggyeong Panjeon is located in a southwestern direction, hence the relative humidity (RH) of the rear is higher and the temperature lower than the RH and temperature at the front. In the case of Beopbojeon, the temperatures and RH at the front and rear of the building ranged from 25.5–26.4℃ to 25.4–25.7℃ and 67.4%–71.7% to 72.2%–73.9%, respectively. During the 2012 survey, the temperature and RH at the front and rear of the building ranged from 22–23.1℃ to 20.5–22.4℃ and from 66.7%–70.3% to 68.4%–74.7%, respectively, showing a similar pattern to the results of this survey. In Sudarajang, the temperature and RH range between now and 2012 were 26.4–27.5℃ and 22.2–23℃ and 66%–70.4%. and 65.6%–70.2%, respectively. The degree of reproduction of fungi on the surface of artifacts is closely related to the temperature and RH of the environment. The management of the RH is the most important factor, and it is generally recommended to keep it within 60% for the conservation of internal environments (Seo et al., 2013).

In Beopbojeon, the deviation in the RH at the front and rear was 2.22 in 2012, increasing to 3.78 in 2021, being a much larger difference. The average temperature and RH values at the front and rear of the Beopbojeon and Sudarajang are presented in Table 1. Overall, the temperature of the Sudarajang is higher and the RH lower than those of Beopbojeon, as shown in Figure 2. The temperature (27.7℃) and RH (64.4%) measured externally in the Beopbojeon and Sudarajang were lower and higher than those of outside. Although this study measured the daytime temperature and RH to determine the difference between the front and rear, other studies have reported the seasonal and monthly average values effect on indoor climate change in the Janggyeong Panjeon (Ahn *et al.*, 1991; Kim *et al.*, 2021). The overall average temperature difference is as low as 0.4°C, which is presumed to be because the indoor space of the Janggyeong Panjeon is directly connected to the outside air through an

Table 1. Average temperature (℃) and relative humidity (%) in the Janggyeong Panjeon in June, 2012 and July, 2021

Site	Year	June 7, 2012		July 23, 2021	
		Temperature	Humidity	Temperature	Humidity
Beopbojeon	Front	22.15	70.38	25.85	69.35
	Rear	21.20	72.60	25.48	73.13
Sudarajang	Front	22.60	67.90	27.45	66.35
	Rear	22.45	68.40	26.75	69.45
Deviation (Front-Rear)	Beopbojeon	0.95	-2.22	0.37	-3.78
	Sudarajang	0.15	-0.50	0.70	-3.10

Measurement time: 14:00～16:00

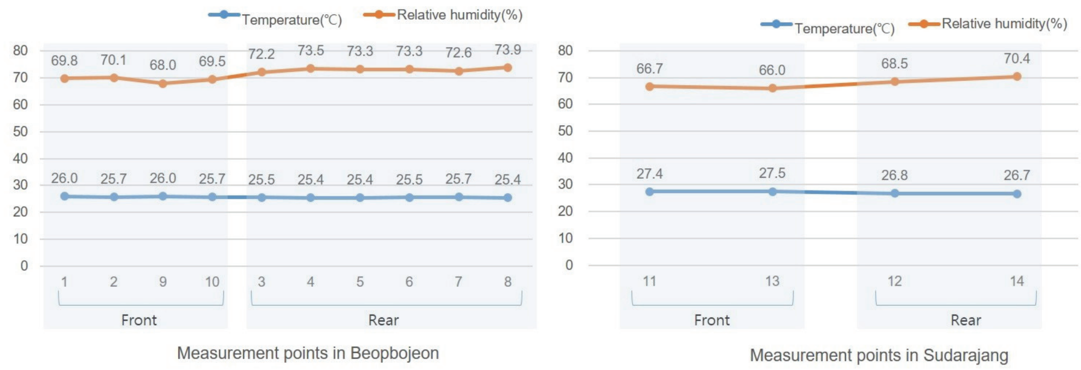

Figure 2. Temperature and relative humidity by measurement point in the janggyeong panjeon.

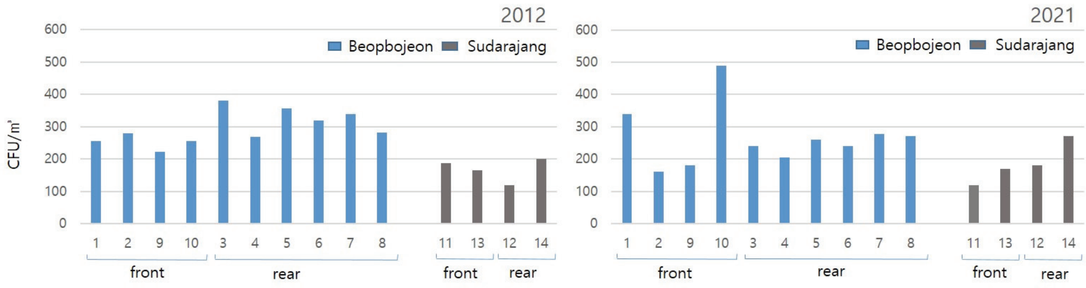

Figure 3. Concentration of airborne fungi by measurement point in the Janggyeong Panjeon.

open window. By contrast, the indoor-outdoor RH differences ranged between 7% and 11%. The average RH throughout the observation period was 61.6% outside and 70.5% inside, with a higher indoor RH than the outdoor RH by 8.9%. These are conducive conditions to the growth of wood-rotting fungi (Kim *et al.*, 2019). Due to its location at the rear end of the precinct, the Janggyeong Panjeon is exposed to air currents blowing from the adjacent mountain slope, which decreases the amount of water vapor in the air rapidly after rainfall. However, due to the lack of such air currents and large amount of wooden material inside the Janggyeong Panjeon, the water vapor absorbed during rainfall is slowly desorbed, resulting in increased humidity.

3.2. Airborne microbial concentration

The Beopbojeon has an area of 530.08 ㎡ (60.47 m × 8.62 m) with the Buddhist sanctuary enshrined in the center (Temple Haeinsa, 2002). Although the internal structure of the Beopbojeon and Sudarajang is similar, the temperature and RH conditions generated by the Beopbojeon's northerly location are expected to favor the growth of fungi. Therefore, the structure was subdivided into three parts and a corner point was added, while the Sudarajang was investigated by dividing the front and rear based on the central shelf.

As a result, when airborne fungi were collected at 10 points of the Beopbojeon, an average of 266.4 CFU/m^3 of colonies were cultured, while an average of 185 CFU/m^3 of colonies were found at four points of the Sudarajang, indicating that the density of airborne fungi inside the Beopbojeon was higher than that at the Sudarajang. When confirming the microbial cluster counts compared with those obtained in 2012, airborne fungi in the Beopbojeon decreased by approximately 7%, whereas they increased by 10% in the Sudarajang. However, checking at each survey point, the number of fungi in air at the front side of the Sudarajang was similar or slightly decreased, while the number of fungi in the air at the rear side increased slightly compared to the previously reported airborne fungi in 2012. The CFU/m^3 from the Beopbojeon and Sudarajang are presented in Figure 3. As shown, the concentration of fungi in the air of the Beopbojeon is generally higher than that of the Sudarajang, and the concentration of airborne fungi in the rear was higher than that at the front. As mentioned earlier, the degree of reproduction of fungi occurring on the surface of relics or in the air is closely related to the temperature and RH of the environment, with the RH potentially making a great difference in the concentration of airborne fungi. It was confirmed that the concentration of airborne fungi is higher in the rear where the RH is high, and the distribution of more airborne fungi in the Beopbojeon, where the average RH is higher than that of the Sudarajang, supports this association (Table 1). The highest concentrations in the Beopbojeon had at 340 and 490 CFU/m^3 in point 1 and 10, respectively. These points showed a very different trend from the results collected in 2012, with a significant difference in the concentrations at other points in the front (points 2 and 9) measured at the same time. It is speculated that such a result may be caused by the uneven movement of air or the flow of wind due to the Buddhist hall located in the center. It seems that these results should be monitored continuously using various methods in the future. Further, it was confirmed that the concentration was 200 CFU/m^3 or more at all other points in the rear. In the case of the Sudarajang, it was 120 and 170 CFU/m^3 at the front (east and west, respectively), and 180 and 270 CFU/m^3 at the rear, more than 50% higher at the rear than that at the front.

The results of the 2012 and 2021 surveys were similar to those of the study by Hong *et al.* (2011). The average result of airborne fungi was reported as 287 CFU/m^3 in August 2008, and the average fungi of 163 and 175 CFU/m^3 for the front and rear were reported for the Sudarajang (it is worth noting that the 100 L was converted to cubic meters). Currently, although there are management regulations for the concentration of indoor airborne fungi in museums and exhibition halls, there is no standard for the protection of cultural properties. NRICH(2009) suggested that airborne bacteria should be kept below 800 CFU/m^3 and airborne fungi below 80 CFU/m^3 as the recommended standard. However, as discussed in the next chapter, since more airborne fungi than airborne bacteria were isolated, if the recommendation of 80 CFU/m^3 or less is used as a criterion, it can be inferred that there is a very high level of microbial contamination at the Janggyeong Panjeon.

3.3. Identification of fungi

Fungi isolated from the Beopbojeon and Sudarajang were identified by sequencing analysis. Consequently, 23 and 22 fungal genera were identified, respectively. The most numerous taxa were *Cladosporium* and *Penicillium,* with the first genus containing the most common indoor and outdoor molds and spores, which are dispersed by wind and may grow on surfaces when wet (Hoog, *et al.*, 2000), while the spores of *Penicillium* are easily released and dispersed into the air. These two fungi were also detected in various studies in 2012, and are known to have high degradation activity of cellulose and xylan (Hong *et al.*, 2011); when the environmental conditions in the Janggyeong Panjeon are favorable for their growth, they can damage the woodblocks. In addition, 10 species of Ascomycetes (*Alternaria*, *Arthrinium*, *Aspergillus*, *Curvularia*, *Lecanicillium*, *Leptosphaerulina*, *Magnaporthe*, *Paecilomyces*, *Periconia*, and *Torula*) and 1 species of Basidiomycota (*Ceriporia*) was commonly present in the air of the Beopbojeon and Sudarajang. However, it was confirmed that the diversity of fungi was different depending on the collection point. In the case of the Beopbojeon, the proportion of *Penicillium* and *Cladosporium* was high at points 1 and 10, respectively. That is, where the concentration of airborne fungi was highest, the dominant mold was different. In addition, several *Cladosporium* were identified at point 4. While many Ascomycetes were cultured at most sites, Basidiomycota such as *Trametes* was dominant at point 9. Several *Penicillium* and *Lecanicillium* were collected from the rear of the west Sudarajang, while Basidiomycota such as *Emmia lacerata* was isolated at a high rate. Basidiomycetes such as *Bjerkander*, *Ceriporia*, *Trametes*, and *Emmia* lacerate are wood-rotting fungi that degrade cellulose, hemicellulose, and lignin (Lee *et al.*, 2007). The airborne fungi isolated in the Beopbojeon and Sudarajang are shown in Table 2.

3.4. Fungal distribution on the Daejanggyeongpan

Fungi were collected from the surface of the Daejanggyeongpan stored in various locations of the

Table 2. Fungi isolated from Janggyeong Panjeon

	Airborne fungi		Daejanggyeongpan	
No.	Species	Sites	Species	Sites
1	*Acremonium* (A)	B	*Alternaria* (A)	B
2	*Alternaria* (A)	B and S	*Arthrinium* (A)	B
3	*Arthrinium* (A)	B and S	*Aureobasidium pullulans* (A)	B
4	*Aspergillus* (A)	B and S	*Bacillus megaterium* (Bac)	B
5	*Bjerkandera* (B)	S	*Botrytis cinerea* (A)	S
6	*Bipolaris* (A)	B	*Coniochaeta africana* (A)	B and S
7	*Ceriporia* (B)	B and S	*Fusarium* (A)	B
8	*Cladosporium* (A)	B and S	*Lecanicillium* (A)	B
9	*Curvularia* (A)	B and S	*Penicillium* (A)	B and S
10	*Dendryphion* (A)	S	*Pestalotiopsis* (A)	B
11	*Emmia lacerata* (B)	S	*Scleroconidioma* (A)	S
12	*Eurotiomycetes* (A)	B	*Sydiwia polyspora* (A)	S
13	*Flavodon* (A)	B	*Trichoderma* (A)	B and S
14	*Hypocrea* (A)	B		
15	*Lecanicillium* (A)	B and S		
16	*Leiotrametes* (B)	B		
17	*Leptosphaerulina* (A)	B and S		
18	*Magnaporthe* (A)	B and S		
19	*Microdochium* (A)	S		
20	*Mycorrhizal* (A)	S		
21	*Nigrospora* (A)	B		
22	*Paecilomyces* (A)	B and S		
23	*Penicillium* (A)	B and S		
24	*Periconia* (A)	B and S		
25	*Paraconiothyrium* (A)	S		
26	*Pithomyces* (A)	S		
27	*Psathyrella* (B)	S		
28	*Torula* (A)	B and S		
29	*Trametes* (B)	B		
30	*Trichoderma* (A)	B		
31	*Xylariaceae* (A)	B		
32	*Vanderbylia* (B)	S		
Total	32 Fungi		12 Fungi and 1 bacterium	

B: Beopbojeon, S: Sudarajang, (A): Ascomycetes, (B): Basidiomycetes, (Bac): Bacterium

Beopbojeon and Sudarajang. The measurement points are shown in Figure 1. First, although a large amount of dust was visible on the surface, no biological damage was observed. However, a large number of fungi were observed on culturing the samples collected from the surface.

There were differences in the clusters of fungi depending on the collection point, but there were no differences between the Beopbojeon and Sudarajang. In total, 12 fungal genera and 1 bacterial genus were identified, and the presence of *Alternaria*, *Penicillium*, and *Trichoderma* was confirmed. Among these, *Trichoderma* showed a high level of lignin-degrading activity, including *Arthrinium* as previously reported (Lee *et al.*, 2018), while *Penicillium* had a high cellulase activity. *Alternaria* was found to dominate at points 1, 5, and 6 in the Beopbojeon, while *Penicillium* was widely distributed in points 3 and 4. *Penicillium* and *Coniochaeta* were mainly distributed at three points in the Sudarajang; *Trichoderma*, *Botrytis cinerea*, and *Scleroconidioma sphagnicola* were also identified. Interestingly, although various *Cladosporium* were collected in the air, they were not detected on the surface of the Daejanggyeongpan. *Alternaria*, *Arthrinium*, *Lecanicillium*, *Penicillium,* and *Trichoderma* were generally identified on the surface of the Daejanggyeongpan and indoor air. *Aureobasidium pullulans*, *Botrytis cinerea*, *Coniochaeta africana*, *Fusarium*, *Sydiwia polyspora*, and *Bacillus megaterium* were only isolated from the surface of the Daejanggyeongpan. Kim *et al.* (2015) conducted a wood discoloration experiment using *A. pullulans* and *Coniochaeta*, and found that *A. pullulans* discolored wood black. Wood is affected by surface contaminants if present for at least 4 weeks under 75% or more RH. *Alternaria, Trichoderma*, and *Penicillium* with *Aspergillus* are classified as surface contaminants that discolor wood surface. *Fusarium* and *Cladosporium* are also sapwood fungi, which do not affect the strength of wood, but do affect the economic value of wood (Kim, 2004; Heo *et al.*, 2007). We found that 58.3% of the fungi identified on the surface were not observed in the indoor air samples. Because the diversity of airborne fungi varies with season and time zone (Ana *et al.*, 2006; Shin, 2015; Kim *et al.*, 2019), surface-dwelling fungi may accumulate dust to form microbial communities over time.

4. CONCLUSION

In this study, the distribution of indoor air fungi of the Janggyeong Panjeon and on the surface of the Daejanggyeongpan was investigated to confirm changes in microbial diversity and distribution between the 2012 and 2021 survey results. The concentration of fungi in the air differed depending on the survey point, in addition to the difference in diversity. Because the diversity of airborne fungi varies with season and time zone, surface-dwelling fungi may form microbial communities with trapped environmental dust. In addition, as mentioned in other studies, the RH inside the Janggyeong Panjeon was similar or slightly higher than that outdoors, with a difference in indoor relative humidity at the front and rear. If these conditions persist, it can be favorable for the generation and propagation of fungi, which can damage the Janggyeong Panjeon and Daejanggyeongpan (woodblocks). The structure of the Janggyeong Panjeon is directly exposed to the external environment; the temperature rises from summer to autumn and the increase in humidity by precipitation are unavoidable adverse conditions. Further, the concentration of airborne fungi confirmed in this survey cannot be compared with the concentration of airborne fungi at the same location surveyed in 2012. Although the Janggyeong Panjeon has a well-equipped ventilation system, changes in the concentration of airborne fungi are likely to occur because the structure is highly influenced by the external environment. A long-term continuous investigation by various research methods is therefore warranted, as efforts to prevent biological damage are required.

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