Pan *et al. Annals of Microbiology* (2023) 73:8
https://doi.org/10.1186/s13213-022-01705-4

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE

Open Access

Analysis of microbial community and biodeterioration of maritime cultural relics (ironware, porcelain, axes, hull wood) from the Nanhai No. 1 shipwreck

Jiao Pan[1†], Yeqing Han[2†], Cen Wang[2], Jing Du[3], Yu Wang[2], Yue Chen[3], Xinduo Huang[2], Kaixuan Ma[2], Zhiguo Zhang[3] and Naisheng Li[3*]

Abstract

Purpose Maritime cultural relics from the Nanhai No. 1 shipwreck were immersed in a buffer to maintain stability. To better monitor the changes in the composition of microorganisms in the buffer and, thus, prevent the damage to artifacts caused by harmful microorganisms.

Methods In September and November 2019, we conducted high-throughput sequencing of water samples from four types of maritime cultural relics (ironware, porcelain, axe, and hull wood) to reveal the composition and changes in microbial communities. In addition, we isolated culturable microorganisms and conducted biocide sensitivity tests and lignin and cellulose degradation tests.

Results Visible microbial colonization was observed in the water samples collected from the buffer solutions of ironware, porcelain, axe, and hull wood of the Nanhai No. 1 shipwreck; additionally, apparent differences in the composition of microorganisms in the water samples collected from different cultural relics and different collection times of the same cultural relics were noted. Few species of bacteria and fungi from the microbial community observed in the maritime cultural relics were cultured, and it was noted that various biocides had certain inhibitory effects on them. Some dominant strains had lignin and cellulose degradation abilities and could only grow under specific environmental conditions.

Conclusion We found apparent differences in the composition of microorganisms obtained from different cultural relics and different collection times of the same cultural relics. This study can provide data support for better protection of maritime cultural relics obtained from the Nanhai No. 1 shipwreck and provide a theoretical basis for the biological protection of other maritime cultural relics.

Keywords Nanhai No. 1 shipwreck, Maritime cultural relics, High-throughput sequencing, Microbial communities, Biodeterioration

[†]Jiao Pan and Yeqing Han equally contributed to this work.

*Correspondence:
Naisheng Li
lineas@126.com
[1] Institute for Cultural Heritage and History of Science & Technology, University of Science and Technology Beijing, Beijing 100083, China
[2] Key Laboratory of Molecular Microbiology and Technology of the Ministry of Education, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin 300071, China
[3] National Centre for Archaeology, Beijing 100013, China

Background

The Nanhai No. 1 is a Chinese wooden merchant ship, which sang into the South China Sea during the Southern Song Dynasty between 1127 and 1279 AD. It was salvaged as a whole in 2007, preserved in the Maritime Silk Road Museum in Guangdong Province, and excavated in 2013 (Cui 2019; Wu and Zhang 2008). To date, more than 180,000 pieces of maritime cultural relics have been unearthed from the Nanhai No. 1 shipwreck, including porcelain, ironware, gold, and other precious relics (Cui 2018).

Owing to the change in environmental temperature and humidity after the unearthing of the cultural relics from the seawater, they were inevitably prone to microbial damage. Maritime cultural relics from the Nanhai No. 1 shipwreck can be divided into wooden hull and ship carrying cultural relics. Under the synergistic action of salt and various microorganisms, wooden cultural relics had undergone serious physical and chemical damage and biodegradation (Hocker 2010; Fors et al. 2012; Fors and Sandström 2006). The original wood components, including cellulose and lignin, were degraded and lost; the moisture content in wood cells had increased, and the supporting force between wood fibers had decreased, resulting in the decay of the structure of wooden cultural relics (Li et al. 1984; Bjordal et al. 1999; Capretti et al. 2008), whereas the ironware was ionized in seawater, and various valences of sulfides were formed under the action of microorganisms, forming insoluble or slightly soluble deposits on the surface and inside the wooden cultural relics. The cultural relics in the Nanhai No. 1 shipwreck are mainly porcelain and few metal. Owing to seawater immersion and biological influence, hard and dense concretions were formed on the surfaces of the porcelain and metal cultural relics, which were difficult to remove. In addition, the soluble salts in porcelain and metal cultural relics were dissolved and recrystallized according to the changes in environmental factors, resulting in various cultural relic diseases.

During the excavation and protection of maritime cultural relics, some biodeterioration problems may arise. Bacteria, fungi, and other microorganisms are a major problem in the process of cultural heritage protection because of their potential for biodeterioration (Sterflinger and Piñar 2013). Different cultural relics may be prone to different microbial colonizations. Wooden cultural relics are rich in nutrients, hence promoting colonization and growth of microorganisms (Eleanor et al. 2008; Björdal 2012). For example, fungal colonization was found on a canoe (Zhang et al. 2019), waterlogged archeological wood found in Sweden showed microbial decay (Bjordal et al. 1999), and wooden relics from the Xiaobaijiao No. 1 were damaged by erosion bacteria and tunneling bacteria (Gao et al. 2017). Metal cultural relics may also undergo biodeterioration and biological corrosion (Hu and Bi 2007; Song et al. 1992; Zhang and Hao 1998; Yu and Yang, 2018; Nugarli and Bartolini, 1997). Zarasvand analyzed typical corrosion parts with pitting and black corrosion products at the bottom of the hull and found a large number of sulfate-reducing bacteria, most of which were typical corrosive bacteria belonging to *Desulfovibrio* sp. (Zarasvand and Rai 2016).

When biodeterioration of cultural relics occurs, measures must be taken to eliminate the damage caused by biodeterioration to the main body of cultural relics. The most commonly used method is the use of safe and green chemical biocides. For example, isothiazolinone biocides, biotin, catechin, and nanoscale zinc oxide have been widely used for the protection of cultural relics (Ji et al. 2016; Jing et al. 2017; Ríos et al. 2012; Polo et al. 2010; Li et al. 2010). At present, the biocide used in Nanhai No. 1 is isothiazolinone biocide Euxyl®K100. Additionally, the detection of biodeterioration is indispensable. The vigorous development of next-generation sequencing technology has led it to be widely used in various biological and medical detection fields (Goodwin et al. 2016; Shokralla et al. 2012). The next-generation sequencing technology combined with traditional microbial isolation and identification could solve these problems efficiently (Ward et al. 1990; Dissanayake et al. 2018; Jayawardena et al. 2018), aiming to better protect and study cultural relics.

Herein, we collected water samples from four different types of maritime cultural relics from the Nanhai No. 1 shipwreck in September and November 2019, including ironware, porcelain, axe, and hull wood (Fig. 1 and Table 1). The microbial community was comprehensively analyzed by optical microscopy, high-throughput sequencing, and traditional microbial isolation and identification methods. Biocide susceptibility tests and lignin and cellulose degradation tests of culturable microorganisms were performed. Our results provide a theoretical basis for the protection of maritime cultural relics in the future.

Results

Microbial diversity analysis by high-throughput sequencing

To reveal the composition of the microbial community, we performed high-throughput sequencing of the microorganisms collected from the water samples of four types of cultural relics. Figure 2A and 2B show the distribution of dominant bacteria in the 12 water samples. Figure 2A shows the distribution of the bacteria at the phylum level. Proteobacteria, at the phylum level, accounted for the largest proportion of all samples; however, its abundance slightly varied, accounting for 47.03–97.38% of the

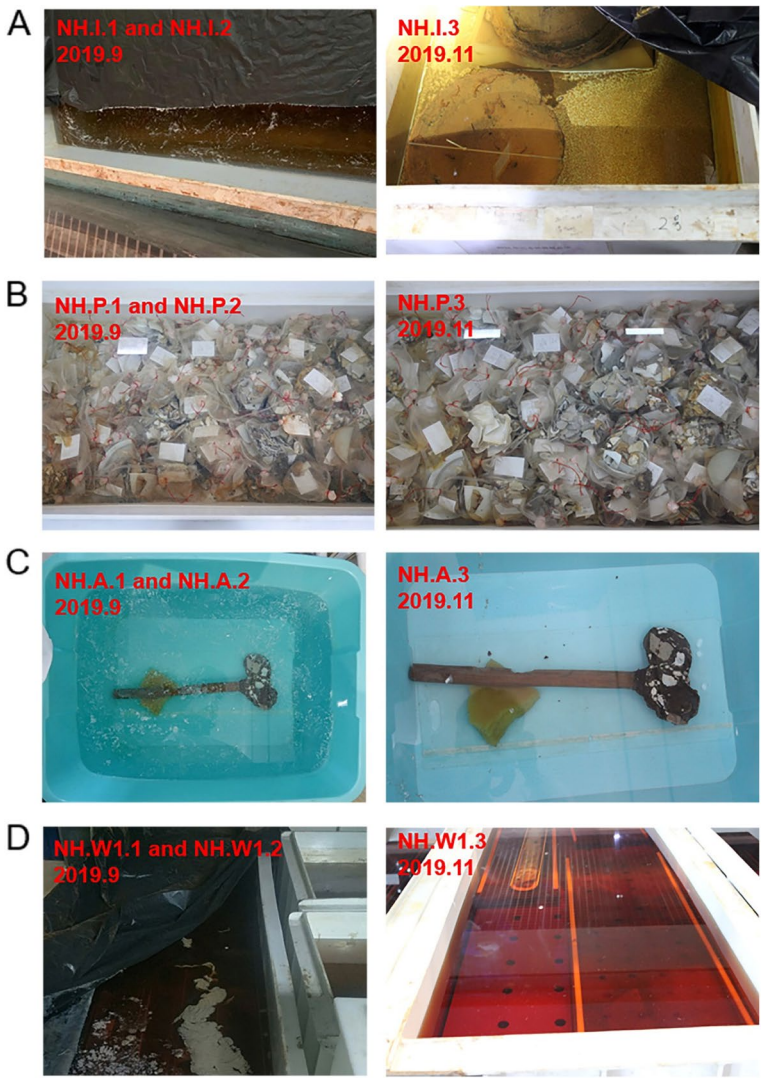

Fig. 1 Sampling pictures of four kinds of maritime cultural relic immersion water samples. Sampled in September 2019 and November 2019, respectively. **A** Ironware. **B** Porcelain. **C** Axe. **D** Hull wood

Table 1 Sampling information of four kinds of maritime cultural relic immersion water samples

Cultural relics	Sample name	Sampling time	Water temperature (℃)	pH	Buffer
Ironware	NH.I.1 and NH.I.2	2019.9	24.7	9.42	Deionized water, containing 5% sodium sesquicarbonate
	NH.I.3	2019.11	22.5	9.53	Deionized water, containing 5% sodium sesquicarbonate and 0.5% Isothiazolinone Euxyl®K100
Porcelain	NH.P.1 and NH.P.2	2019.9	20.1	7.82	Deionized water
	NH.P.3	2019.11	20.9	6.86	Deionized water
Axe	NH.A.1 and NH.A.2	2019.9	26.3	7.73	Deionized water
	NH.A.3	2019.11	21.3	6.65	Deionized water
Hull wood	NH.W1.1 and NH.W1.2	2019.9	24.5	6.83	Deionized water, containing 0.7‰ Isothiazolinone Euxyl®K100 and 10 mmol/L EDTA-2Na
	NH.W1.3	2019.11	20.6	5.65	Deionized water, containing 0.5% Isothiazolinone Euxyl®K100 and 10 mmol/L EDTA-2Na

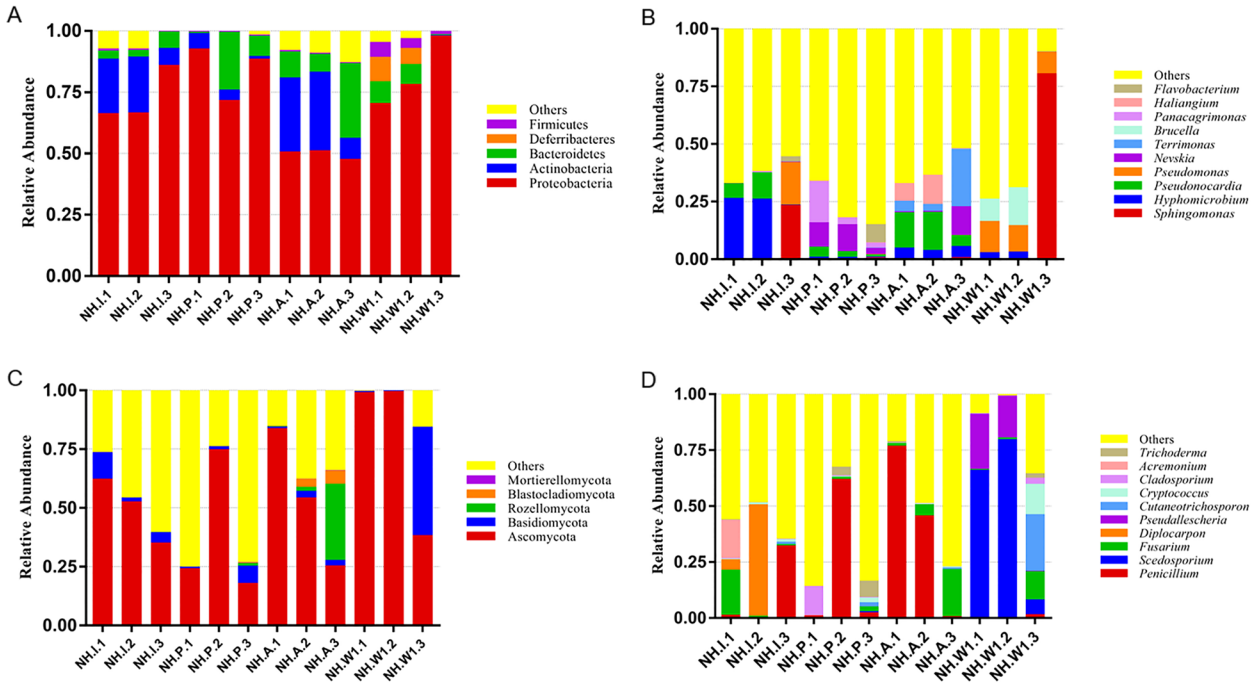

Fig. 2 The relative abundance of microbial communities in four kinds of maritime cultural relic immersion water samples. The relative abundance is shown as a percentage. Phylum and genera are colored according to the legend on the right. **A** Relative abundance of bacteria in water samples at the phylum level. **B** Relative abundance of bacteria in water samples at the genera level. **C** Relative abundance of fungi in water samples at the phylum level. **D** Relative abundance of fungi in water samples at the genera level

community. The samples also contained Actinobacteria and Bacteroidetes. Figure 2B and Table S1 show the distribution of the bacteria at the genus level. The composition of bacteria, at the genus level, in each water sample was significantly different. The two parallel water samples of the same cultural relic collected in September were similar and were evidently different from the one collected in November. Figure 2C and 2D show the distribution of the dominant fungi in the 12 water samples. High-throughput results for fungi in water samples NH. W1.1 and NH. W1.2 were obtained from a previous study (Han et al. 2021). Figure 2C shows the distribution of the fungi at the phylum level. Ascomycota was dominant at the phylum level in 10 samples; however, the abundance slightly varied. In addition, Rozellomycota and Basidiomycota were dominant in NH.A.3 and NH.W1.3, respectively. Figure 2D and Table S2 show the distribution of fungi at the genus level. We found significant differences in the composition of fungi in different cultural relics and different collection times of the same cultural relics.

Among the three ironware water samples, the microbial composition in the two parallel water samples (NH.I.1 and NH.I.2) collected in September was relatively similar, and *Hyphomicrobium* was the most abundant bacteria, accounting for 25.90% and 25.51%, respectively. One water sample (NH.I.3) collected in November was significantly different from that collected in September, and the most abundant bacteria were *Sphingomonas*, accounting for 22.89%. Comparing the three ironware water samples, we found a significant difference between NH.I.1, NH.I.2, and NH.I.3 (fungi). Among them, the most abundant fungi were *Fusarium*, *Diplocarpon*, and *Penicillium*, accounting for 20.17%, 49.79%, and 31.67%, respectively.

Among the three porcelain water samples, the microbial composition in the two parallel water samples (NH.P.1 and NH.P.2) collected in September slightly differed. Among them, the most abundant bacteria were *Panacagrimonas* and *Nevskia*, accounting for 17.91% and 11.67%, respectively. One water sample (NH.P.3) collected in November significantly differed from that collected in September, and the most abundant bacteria were *Flavobacterium*, accounting for 7.84%. The most abundant fungi in NH.P.1, NH.P.2, and NH.P.3 were *Cladosporium*, *Penicillium*, and *Trichoderma*, accounting for 12.99%, 61.36%, and 7.26%, respectively.

Among the three axe water samples, the microbial composition in the two parallel water samples (NH.A.1 and NH.A.2) collected in September was relatively similar, and the most abundant bacteria were *Pseudonocardia*, accounting for 15.39% and 16.51%, respectively. One water sample (NH.A.3) collected in

November significantly differed from that collected in September, and the most abundant bacteria were *Terrimonas*, accounting for 24.84%. In contrast, the two parallel water samples (NH.A.1 and NH.A.2) collected in September were relatively similar, and the most abundant fungi were *Penicillium*, accounting for 76.22% and 45.11%, respectively. One water sample (NH.A.3) collected in November significantly differed from that collected in September, and the most abundant fungi were *Fusarium*, accounting for 20.99%.

Among the three hull wood samples, the bacterial composition in the two parallel water samples (NH.W1.1 and NH.W1.2) collected in September slightly differed. Among them, the most abundant bacteria were *Pseudomonas* and *Brucella*, accounting for 13.57% and 16.41%, respectively. One water sample (NH.W1.3) collected in November significantly differed from that collected in September, and the most abundant bacteria were *Sphingomonas*, accounting for 79.97%. Among the three hull wood water samples, the fungal composition in the two parallel water samples (NH.W1.1 and NH.W1.2) collected in September was relatively similar, and the most abundant fungi were *Scedosporium*, accounting for 65.45% and 79.10%, respectively. One water sample (NH.W1.3) collected in November was significantly different from that collected in September, and the most abundant fungi were *Cutaneotrichosporon*, accounting for 25.26%.

These results show that there were significant differences in the composition of microorganisms in different cultural relics and different collection times of the same cultural relics.

Isolation and identification of culturable microorganisms

Maritime cultural relic immersion water samples were evenly applied to lysogeny broth (LB) and potato dextrose agar (PDA) media after gradient dilution. After culturing, purification, and identification, five culturable bacteria and fungi were isolated (Table 2). The single colony morphology and microstructure (mycelia and spores) of these fungi were observed and recorded using an optical microscope (Fig. 3A–E).

The susceptibility test of culturable fungi and dominant bacteria to biocides

Five culturable fungi were obtained from maritime cultural relic immersion water samples. Among the five culturable bacteria, the high-throughput results showed that *Pseudomonas* sp. (NH.W1-B1) was the only dominant bacteria. Therefore, we used the filter paper diffusion method to conduct biocide susceptibility tests on five culturable fungi and one dominant bacteria. The inhibitory effects of different biocides vary based on the strain of bacteria and fungi (Fig. 4). Different biocides had a certain inhibitory effect on the five fungi, among which D7 was the best, and P91 and K100 were inferior. The results showed that P91 and 20 N inhibited *Pseudomonas* sp. (NH.W1-B1), while K100 and D7 had no inhibitory effect.

Ligninolytic and cellulolytic enzymatic activity of *Pseudomonas* sp. (NH.W1-B1)

We conducted lignin and cellulose degradation tests on the dominant bacteria *Pseudomonas* sp. (NH.W1-B1). We found that in the sodium lignosulfonate agar

Table 2 Molecular identification of culturable strains isolated from four kinds of water samples

Bacteria	Closet relative strain	Phylum	Similarity (%)	Accession number	Source
NH.P-B1	*Staphylococcus epidermidis*	Firmicutes	100%	MN511770.1	Porcelain water sample
NH.P-B2	*Burkholderia* sp.	Proteobacteria	99%	MN263850.1	Porcelain water sample
NH.P-B3	*Bacillus subtilis*	Firmicutes	100%	MG705979.1	Porcelain and axe water sample
NH.W1-B1	*Pseudomonas* sp.	Proteobacteria	100%	JQ660555.1	Hull wood water sample
NH.W1-B2	*Ochrobactrum* sp.	Proteobacteria	100%	KJ127515.1	Hull wood water sample
Fungi	**Closet relative strain**	**Phylum**	**Similarity (%)**	**Accession number**	**Source**
NH.I-F1	*Cladosporium* sp.	Deuteromycotina	98%	MH325932.1	Ironware water sample
NH.I-F2	*Cladosporium* sp.	Deuteromycotina	98%	MG746382.1	Ironware water sample
NH.I-F3	*Cladosporium* sp.	Deuteromycotina	99%	HM535372.1	Ironware and axe water sample
NH.P-F1	*Penicillium* sp.	Ascomycota	98%	KJ001170.1	Porcelain water sample
NH.A-F1	*Cladosporium* sp.	Deuteromycotina	99%	MH884146.1	Axe water sample
NH.W1-1	*Penicillium* sp.	Ascomycota	100%	GU212865.1	Hull wood water sample [39]
NH.W1-2	*Penicillium citrinum*	Ascomycota	100%	MN398977.1	Hull wood water sample [39]
NH.W1-3	*Scedosporium apiospermum*	Ascomycota	100%	FJ713053.1	Hull wood water sample [39]
NH.W1-4	*Fusarium solani*	Ascomycota	100%	MN066126.1	Hull wood water sample [39]
NH.W1-5	*Cladosporium* sp.	Deuteromycotina	99%	MN265985.1	Hull wood water sample [39]

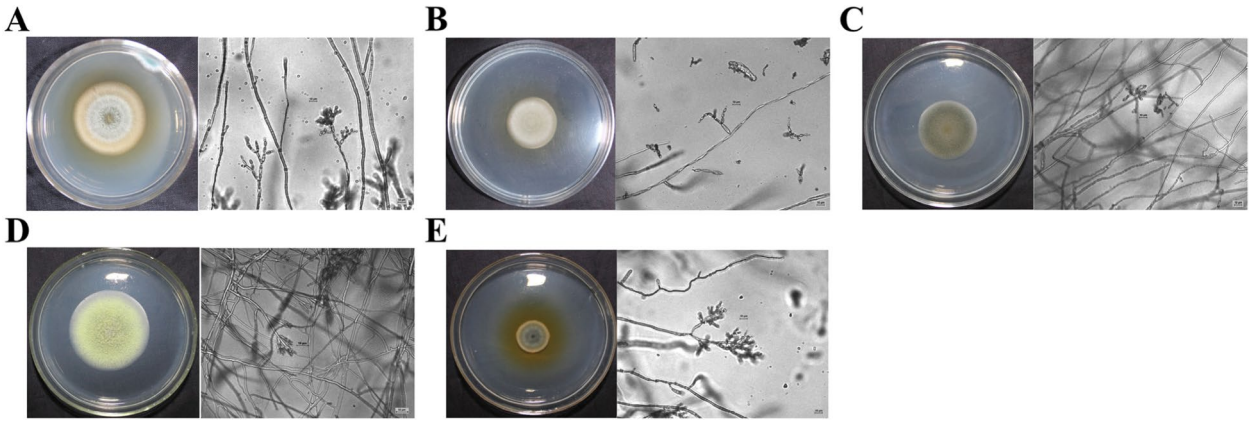

Fig. 3 Single colony morphology and microstructure (mycelia and spores) of culturable fungi from water samples. The fungi were inoculated onto the PDA medium and incubated at 28° C for 3 days. The scale is 10 μm. **A** *Cladosporium* sp. (NH.I-F1). **B** *Cladosporium* sp. (NH.I-F2). **C** *Cladosporium* sp. (NH.I-F3). **D** *Penicillium* sp. (NH.P-F1). **E** *Cladosporium* sp. (NH.A-F1). Bar = 10 μm

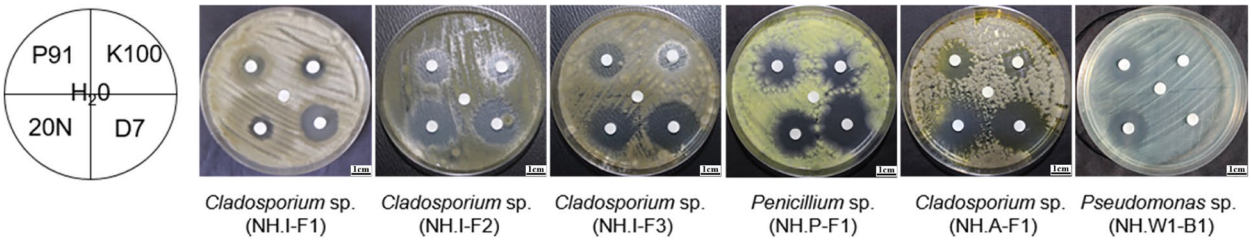

Fig. 4 The sensitivity of culturable fungi and dominant bacteria to biocides. The size of the inhibition zone indicates the inhibitory effect of biocides on culturable strains. The fungi were cultured at 28℃ for 3 days, and the bacteria were cultured at 37℃ for 1 day

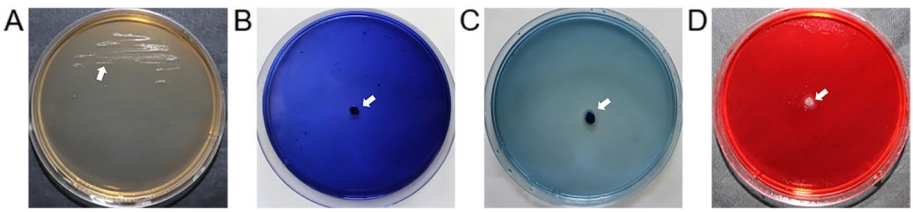

Fig. 5 **A** *Pseudomonas* sp. (NH.W1-B1) could grow on the sodium lignosulfonate agar medium. **B** Decolorizing circles of *Pseudomonas* sp. (NH.W1-B1) grown on the aniline blue agar medium for 5 days. **C** Decolorizing circles of *Pseudomonas* sp. (NH.W1-B1) grown on the remazol brilliant blue agar medium for 5 days. **D** Decolorizing circles of *Pseudomonas* sp. (NH.W1-B1) grown on the CMC-Na agar medium for 5 days

medium with only sodium lignosulfonate as the carbon source, *Pseudomonas* sp. can be grown using sodium lignosulfonate (Fig. 5A), whereas on an aniline blue agar medium and a remazol brilliant blue agar medium, decolorizing circles appeared around *Pseudomonas* sp. (NH.W1-B1) (Fig. 5B, C), indicating that it can produce lignin-degrading enzymes. In addition, in the CMC-Na agar medium, after staining with Congo red, a visible decolorizing circle (Fig. 5D) was observed, indicating that *Pseudomonas* sp. (NH.W1-B1) can produce cellulose-degrading enzymes. Therefore, we concluded that *Pseudomonas* sp. (NH.W1-B1) had lignin and cellulose degradation abilities.

Discussion

Herein, we combined high-throughput sequencing technology with traditional microbial isolation and identification methods to detect and explore the biodeterioration

of four types of maritime cultural relic immersion water samples from the Nanhai No. 1 shipwreck. Through comprehensive detection of microorganisms, we evaluated the preservation status of maritime cultural relics and provided data support for solving biological problems. First, we analyzed the water samples using an optical microscope and observed several fungi-like microorganisms, indicating evident microbial colonization. We then performed high-throughput sequencing and traditional microbial isolation and identification. The results showed that among the five strains of culturable bacteria, *Pseudomonas* sp. (NH.W1-B1) was the only dominant bacteria in the hull wood water samples. Additionally, through lignin and cellulose degradation tests, we found that *Pseudomonas* sp. (NH.W1-B1) had certain lignin and cellulose degradation abilities. Furthermore, the biocide sensitivity test showed that biocides P91 and 20 N had inhibitory effects on *Pseudomonas* sp. (NH.W1-B1). Furthermore, all the five culturable fungi accounted for a certain proportion of the water samples. In addition, culturable fungi were sensitive to isothiazolinone biocides. Through the combination and comparison of the data from high-throughput sequencing and traditional microbial isolation and identification, we drew the following conclusions: (1) there were significant differences in the composition of microorganisms in water samples from different cultural relics; (2) there were significant differences in the composition of microorganisms in water samples from the same cultural relics at different collection times; (3) there were few species of bacteria and fungi that could be cultured, and some dominant strains may only grow under specific environmental conditions.

There were certain differences in the biodeterioration of the different maritime cultural relics. In this study, ironware and axes were metal cultural relics containing iron. Maritime metal cultural relics have concretions that are difficult to remove and exhibit a high degree of corrosion (Wan 2019). When maritime metal cultural relics are salvaged, they need to be rapidly cleaned, rust removed, immersed in a buffer, and moisturized. After stabilization, they were subjected to subsequent treatments. The ironware used in this study was immersed in deionized water containing sodium sesquicarbonate and biocide. The pH of the water samples in September and November exceeded 9.0, which was alkalescent, and the average water temperature was 23.6℃. The axe relics were immersed in deionized water. The pH of the water samples in September and November was approximately neutral, and the average water temperature was 23.8℃. The high-throughput results showed that at the phylum level, although ironware and axe relics were dominated by Proteobacteria, similar to other cultural relic water samples, the proportion of Proteobacteria was significantly lower and Actinobacteria was significantly higher than that of other cultural relic water samples. At the bacterial genus level, the major bacterial species found in ironware water samples were *Hyphomicrobium*, *Pseudonocardia*, *Sphingomonas*, and *Pseudomonas* and in axe water samples were *Pseudonocardia*, *Nevskia*, and *Terrimonas*. At the genus level, three ironware and one axe water samples were dominated by *Ascomycota* and one axe water sample was dominated by *Rozellomycota*. At the fungal genus level, the major fungal species in ironware water samples were *Fusarium*, *Diplocarpon*, *Penicillium*, and *Acremonium* and in the axe water samples were *Penicillium* and *Fusarium*. Most of the dominant bacteria in the ironware and axe water samples grew over a wide range. Some bacteria can participate in the metabolism of sulfur and iron, such as *Hyphomicrobium* (Haaijer et al. 2008), *Pseudonocardia* (Pan et al. 2015), and *Pseudomonas* (Sultan and Faisal 2016). Some dominant bacteria tend to live in a polluted environment, such as *Sphingomonas* (Leys et al. 2004), *Nevskia* (Hao et al. 2009), and *Terrimonas* (Meng et al. 2020). The dominant fungal species in ironware and axe water samples have a certain heavy metal (Rasool and Irum 2014a, b; Rasool and Irum 2014a, b) and high salt tolerance (Grishkan and Nevo 2003). *Fusarium* can metabolize sulfur and iron (Etemadzadeh et al. 2016), and *Penicillium* often colonizes cultural relics (Zhang et al. 2019). At present, research on the biodeterioration of cultural relics is mostly focused on wooden cultural relics (Zhang et al. 2019; Liu et al. 2017), mural cultural relics (Ma et al. 2020; Suphaphimol et al. 2022), and stone cultural relics (Zhang et al. 2019), with few studies on metal cultural relics. However, we believe that in the process of excavation and protection of metal cultural relics, the problem of biodeterioration cannot be ignored. Additionally, microorganisms that can metabolize sulfur and iron may appear during the preservation process of some iron-containing cultural relics.

Among all maritime cultural relics unearthed from the Nanhai No. 1 shipwreck, porcelain was the most abundant. After porcelain is unearthed from the seawater, it needs to be immersed in deionized water for static desalination after removing the surface concretion. Hence, the porcelain used in this study was immersed in deionized water. The pH of the water samples in September and November was approximately neutral, and the average water temperature was 20.5℃. The high-throughput results showed that *Proteobacteria* was dominant at the phylum level. At the genus level, the major bacterial species were *Panacagrimonas*, *Nevskia*, and *Flavobacterium*. At the phylum level, *Ascomycota* was dominant. At the genus level, the major fungal species detected were *Cladosporium*, *Penicillium*, and *Trichoderma*. Thus, the dominant bacteria in porcelain water samples, such as

Panacagrimonas (Xue 2015), *Nevskia* (Hao et al. 2009), and *Flavobacterium* (high salt tolerance) (Yoon et al. 2010) (Li et al. 2019), can be isolated from polluted environments. The dominant fungal species in porcelain water samples are often found to colonize the surface of cultural relics (Zhang et al. 2019; Zhang et al. 2019) and have a high salt tolerance (Mbarki et al. 2017). Porcelain itself is not an organic cultural relic and is not easy to corrode and oxidize. Overall, the preservation conditions are relatively stable. However, owing to its extraordinary historical significance and value, its protection requires attention.

The Nanhai No. 1 shipwreck is an ancient wooden ship, and although tens of thousands of precious cultural relics have been unearthed, its maritime wooden cultural relics are considered the most important and have the utmost research and protection value. For some of the wooden cultural relics scattered from the shipwreck, the concretion should first be cleaned and then immersed in deionized water for moisturization, stabilization, and preliminary desalination treatment. The hull wood relic in this study was immersed in deionized water containing EDTA-2Na and a biocide. The pH of the water samples in September and November was weakly acidic, and the average water temperature was 22.6℃. The high-throughput results showed that *Proteobacteria* was dominant at the phylum level. At the genus level, the major bacterial species were *Pseudomonas*, *Brucella*, and *Sphingomonas*. At the phylum level, two water samples were dominated by *Ascomycota* and one water sample was dominated by *Basidiomycota*. At the fungal genus level, the main fungal species detected were *Scedosporium*, *Pseudallescheria*, and *Cutaneotrichosporon*. Thus, the dominant bacteria in hull wood water samples can grow in acidic and high-salt environments (Dhail 2012; Li et al. 2015; Li et al. 2018). Additionally, most of the dominant bacteria and fungi can be isolated from polluted environments (Leys et al. 2004; Obayori et al. 2008; Cycon et al. 2016; Rougeron et al. 2015). They also have the ability to degrade lignocellulose, such as *Pseudomonas* (Yang et al. 2018), *Sphingomonas* (Masai et al. 1999), *Scedosporium* (Han et al. 2021), and *Cutaneotrichosporon* (Yaguchi et al. 2020). The lignin and cell degradation tests showed that the major bacterial species *Pseudomonas* (NH.W1-B1) had the ability to degrade lignin and cellulose. Therefore, more attention should be paid to the *Pseudomonas* genus. As the Nanhai No. 1 is a large ancient maritime wooden ship, the growth of microorganisms possibly had an irreversible impact on its wooden structure during the long-term excavation process, which needs greater attention.

Hence, the protection of maritime cultural relics should be multifaceted. Biological issues need to be highly valued in addition to conventional cultural relic protection. For the biological issues of cultural relics, we need to regularly monitor biodeterioration and take targeted protection measures by observing and studying changes in microbial composition. Additionally, deionized water should be used for immersion in cultural relics, the ion concentration and conductivity of water should be monitored regularly, and the deionized water should be replaced in time to prevent the accumulation of ions and nutrients. Finally, the use of biocides is important for controlling biodeterioration. However, the use of biocides involves a series of problems, such as drug resistance and health (Glécia et al. 2018; Kathiravan et al. 2012), so biocides need to be carefully selected and used.

Conclusions

In summary, by analyzing the composition of microbial communities and detecting damages caused by biodeterioration in the four typical maritime cultural relics in the Nanhai No. 1 shipwreck, we achieved a comprehensive conclusion regarding the biological protection of the cultural relics in the Nanhai No. 1 shipwreck. We found significant differences in the composition of microorganisms in different cultural relics and different collection times of the same cultural relics. Employing and combining high-throughput sequencing technology with traditional microbial isolation and identification methods is crucial to comprehensively detect biodeterioration. Our research provides data support for better protection of maritime cultural relics in the Nanhai No. 1 shipwreck and a theoretical basis for the biological protection of other maritime cultural relics.

Materials and methods

Sample collection

The research objects were four types of maritime cultural relics: ironware, porcelain, axe, and hull wood, which were immersed in a buffer. The Maritime Silk Road Museum, Yangjiang City, Guangdong Province, the archeological excavation site of the Nanhai No. 1 shipwreck, was the sample collection site. Before immersion, the concretions on the surface of the cultural relics were thoroughly cleaned. Samplings were performed in September and November 2019. In September, we collected two 50-mL parallel water samples from each of the four types of cultural relic tanks, labeled NH.I.1 and NH.I.2 (from ironware), NH.P.1 and NH.P.2 (from porcelain), NH.A.1 and NH.A.2 (from axe), and NH.W1.1 and NH.W1.2 (from hull wood). In November, we collected one 50-mL water sample from each of the four types of cultural relic tanks, labeled NH.I.3, NH.P.3, NH.A.3, and NH.W1.3 (from ironware, porcelain, axe, and hull wood, respectively). The sampling pictures and information of

the immersed water samples of the four types of maritime cultural relics are shown in Fig. 1 and Table 1. We used 50-mL aseptic centrifuge tubes to collect water samples, followed by cryopreservation and transport to the laboratory for subsequent experiments.

Microscopic analysis

We used an optical microscope (Nikon E200, Japan) to observe the diluted water samples and recorded them under a 400 × and 1000 × microscope. Additionally, the microstructures of mycelia and spores of culturable fungi were observed using an optical microscope and recorded under a 400 × microscope.

Total DNA extraction and high-throughput sequencing

We extracted total DNA from the 12 water samples. We filtrated 50-mL water samples through a 0.22-μm size filter membrane and used the DNeasy PowerWater Kit (QIAGEN, Germany) to extract the total DNA from the filter membrane. Total DNA was sent to Novogene Genome Sequencing Company. The S5-16SV4 gene region was amplified with the primers F: GTGCCA GCMGCCGCGGTAA and R: GGACTACHVGGG TWTCTAAT for bacteria, and the S5-ITS1-5F region was amplified with the primers F:GCGGTAATTCCA GCTCCAA and R:AATCCRAGAATTTCACCTCT for fungi. First, splice and quality control of the offline data (raw PE) using the Illumina Novaseq sequencing platform were performed to obtain clean tags, followed by chimera filtering to obtain effective tags that can be used for subsequent analysis. The effective tags of all samples were clustered by operational taxonomic units (OTUs) with 97% identity, and the sequences of OTUs were annotated. Based on the results of species annotation, we selected the top 10 species with the highest abundance in each sample or group at each classification level (phylum, genus, and disciplines) and generated a cylindrical accumulation diagram of the relative abundance of species. Thus, the community composition of bacteria and fungi in the water samples was analyzed using high-throughput sequencing.

Isolation and identification of culturable microorganisms

To isolate culturable microorganisms from the water samples, we prepared LB and PDA media to culture and isolate bacteria and fungi, respectively. First, the water samples were diluted in a gradient, and then 10^{-2} and 10^{-3} dilutions were evenly applied to the surface of the LB and PDA media, respectively; these media were cultured at 37℃ and 28℃, respectively, for 1–5 days. We then isolated and purified bacteria and fungi of different sizes, colors, and shapes. After repeating the isolation and purification step for 1–3 times, we obtained pure cultures of bacteria and fungi and stored them on LB and PDA agar slant culture media at 4 °C. In order to identify the species of bacteria, we first extracted the DNA from pure bacterial cultures (Abdellaoui et al. 2011) and then used 341F/907R primers to amplify the 16S rRNA region of bacteria (Yin et al. 2019). A band of approximately 600 bp in size was obtained. To identify the fungal species, we used the T5 Direct PCR Mix kit (TSINGKE, China) to amplify the fungal culture (Han et al. 2021). The primers used were ITS1/ITS4 (White et al. 1990), and a band of approximately 600 bp in size was obtained. The PCR products of the above bacteria and fungi were sent to GENEWIZ (Beijing, China) for Sanger sequencing, and the base sequences obtained were compared with the NCBI databases to determine the bacterial and fungal species. Raw sequencing data were downloaded from the NCBI's Sequence Read Archive database (study accession number: PRJNA708180).

Susceptibility test of culturable microorganisms to biocides

Isothiazolinone has been used for cultural relic protection to inhibit the growth of microorganisms. We selected four types of isothiazolinone derivatives, namely Preventol®D7 (Lanxess, Germany), Preventol®BIT 20 N (Lanxess, Germany), Preventol®P91 (Lanxess, Germany), and Euxyl®K100 (Schulke, Germany) to test the sensitivity of the isolated culturable microorganisms to biocides; the filter paper diffusion method was used to test the sensitivity. First, fungal and bacterial cultures were inoculated evenly in PDA and LB media, respectively. Five 3-layer aseptic filter papers with a diameter of 7 mm were placed on the media. Next, 15 μL of four different biocides (0.5%) was added to the filter paper, and distilled water was added to the middle filter paper as a negative control. The PDA and LB media were cultured at 28℃ for 3 days and 37℃ for 1 day, respectively. The inhibitory effects of different biocides on different culturable microorganisms were observed and compared. The larger the diameter of the inhibition zone, the higher is the sensitivity of culturable microorganisms to biocides (Zhang et al. 2019). The biocide susceptibility test was repeated thrice.

Ligninolytic and cellulolytic enzymatic activity of dominant bacteria

Different media were used to identify the cellulose and lignin degradation ability of the dominant bacteria. We prepared four types of media: sodium lignosulfonate agar medium, aniline blue agar medium, remazol brilliant blue agar medium, and CMC-Na agar medium (Yin et al. 2019). The ability to decompose cellulose and lignin was

evaluated by observing the size of the decolorizing circle after culturing at 37℃ for 5 days and marking whether the strain could grow (Yin et al. 2019).

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-022-01705-4.

Additional file 1: Supplementary Table 1. Relative abundance of dominant bacteria among four kinds of maritime cultural relic immersion water samples at the genus level. **Supplementary Table 2.** Relative abundance of dominant fungi among four kinds of maritime cultural relic immersion water samples at the genus level

Acknowledgements
We gratefully acknowledge the assistance of Prof. Hong Guo from the Institute for Cultural Heritage and History of Science & Technology, University of Science and Technology Beijing.

Authors' contributions
Jiao Pan: conceptualization, methodology, and writing—reviewing and editing.
Yeqing Han: data curation and writing—original draft preparation.
Cen Wang, Yu Wang, Kaixuan Ma, and Xinduo Huang: software, data curation, and validation.
Zhiguo Zhang, Jing Du, and Yue Chen: resources.
Naisheng Li: designed the whole project.
The authors read and approved the final manuscript.

Funding
This work was supported by the Natural Science Foundation of Tianjin #1 under Grant 19JCZDJC33700 and the National Key R&D Program of China #2 under Grant 2020YFC1521805.

Availability of data and materials
The raw sequencing data could be downloaded at the NCBI Sequence Read Archive (SRA) with the study accession number PRJNA708180.
Table S1: Relative abundance of dominant bacteria among four kinds of maritime cultural relic immersion water samples at the genus level. Table S2: Relative abundance of dominant fungi among four kinds of maritime cultural relic immersion water samples at the genus level.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 25 May 2022 Accepted: 28 November 2022
Published online: 03 February 2023

References

Abdellaoui R, Gouja H, Sayah A, Neffati M (2011) An efficient DNA extraction method for desert Calligonum species. Biochem Genet 49(11–12):695–703. https://doi.org/10.1007/s10528-011-9443-7
Bjordal CG, Nilsson T, Daniel G (1999) Microbial decay of waterlogged archaeological wood found in Sweden - applicable to archaeology and conservation. Int Biodeterior Biodegrad 43(1–2):63–73. https://doi.org/10.1016/S0964-8305(98)00070-5
Björdal CG (2012) Microbial degradation of waterlogged archaeological wood. J Cult Herit 13:S118–S122. https://doi.org/10.1016/j.culher.2012.02.003
Capretti C, Macchioni N, Pizzo B, Galotta G, Giachi G, Giampaola D (2008) The characterization of waterlogged archaeological wood: the three Roman ships found in Naples (Italy). Archaeometry 50(5):855–876. https://doi.org/10.1111/j.1475-4754.2007.00376.x
Cui J (2018) The mystery of the 800-year shipwreck of Nanhai No.1. Travel World 004:55–59
Cui Y (2019) Brief summery on excavation of Nanhai No1 shipwreck. Study Nat Cultural Heritage 4(10):14–20
Cycon M, Mrozik A, Piotrowska-Seget Z (2016) Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: a review. Chemosphere 172(APR):52–71. https://doi.org/10.1016/j.chemosphere.2016.12.129
Da-Wa S, Li-Zhi M, Lu P, Qing-Lin M (2009) Coatings materials used for conservation of iron relics. Paint Coatings Ind 39(1):17–19
de los Ríos A, PérezOrtega S, Wierzchos J, Ascaso C (2012) Differential effects of biocide treatments on saxicolous communities: case study of the Segovia cathedral cloister (Spain). Int Biodeterior Biodegradation 67:64. https://doi.org/10.1016/j.ibiod.2011.10.010
Dhail S (2012) Isolation of potent biosurfactant producing bacteria from oil spilled marine water and marine sediments. Afr J Biotech 11(103):16751–16757. https://doi.org/10.5897/AJB12.1510
Dissanayake AJ, Purahong W, Wubet T, et al (2018) Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (Vitis vinifera). Fungal Divers 90:85–107. https://doi.org/10.1007/s13225-018-0399-3
Eleanor TL, Mitchella JI, Hotchkissa S, Eatona RA (2008) Bacterial diversity associated with archaeological waterlogged wood: ribosomal RNA clone libraries and denaturing gradient gel electrophoresis (DGGE). Int Biodeterior Biodeg-Radation 61(1):106–116. https://doi.org/10.1016/j.ibiod.2007.07.007
Etemadzadeh SS, Emtiazi G, Etemadifar Z (2016) Heterotrophic bioleaching of sulfur, iron, and silicon impurities from coal by Fusarium oxysporum FE and Exophiala spinifera FM with growing and resting cells. Curr Microbiol 72(6):707–715. https://doi.org/10.1007/s00284-016-1008-x
Fors Y, Sandström M (2006) Sulfur and iron in shipwrecks cause conservation concerns. Chem Soc Rev 35(5):399–415. https://doi.org/10.1039/b507010b
Fors Y, Jalilehvand F, Risberg ED, Björdal C, Phillips E, Sandström M (2012) Sulfur and iron analyses of marine archaeological wood in shipwrecks from the Baltic Sea and Scandinavian waters. J Archaeol Sci 39(7):2521–2532. https://doi.org/10.1016/j.jas.2012.03.006
Gao Meng-Ge, Zhang Qin-Fen, Jin Tao, Luo P, Li Q, Xu RL (2017) Observation and damage assessment of microbial diseases in some wooden cultural relics from the ancient marine shipwreck, Ningbo Xiaobaijiao No.1. Sci Conserv Archaeol 29(06):102–111
Glécia L, Sousa B, Guedes A, Barreto C, Brasil L (2018) Biocides used as additives to biodiesels and their risks to the environment and public health: a review. Molecules 23(10):2698. https://doi.org/10.3390/molecules23102698
Goodwin S, Mcpherson JD, Mccombie WR (2016) Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 17(6):333–351. https://doi.org/10.1038/nrg.2016.49
Grishkan I, Nevo E, Wasser SP (2003) Soil micromycete diversity in the hypersaline dead sea coastal area. Israel. Mycological Progress 2(1):19–28. https://doi.org/10.1007/s11557-006-0040-9
Haaijer S, Harhangi HR, Meijerink BB, Strous M, Pol A, Smolders AJ (2008) Bacteria associated with iron seeps in a sulfur-rich, neutral pH, freshwater ecosystem. Isme J 2:1231–1242. https://doi.org/10.1038/ismej.2008.75
Han Y, Huang X, Wang Y, Du J, Ma K, Chen Y (2021) Fungal community and biodeterioration analysis of hull wood and its storage environment of the Nanhai No. 1 Shipwreck. Front Microbiol 11:609475.https://doi.org/10.3389/fmicb.2020.609475
Hao C-B, Wang G-C, Dong J-N, Zhang Q, Cai W-T (2009) Bacterial molecular ecology in the groundwater contaminated by oil. Earth Sci Front 16(4):389–400
Heyrman J, Mergaert J, Denys R, Swings J (2010) The use of fatty acid methyl ester analysis (FAME) for the identification of heterotrophic bacteria present on three mural paintings showing severe damage by

microorganisms. FEMS Microbiol Lett 1:55–62. https://doi.org/10.1111/j.1574-6968.1999.tb08826.x
Hocker E (2010) From the micro- to the macro-: managing the conservation of the warship. Vasa Macromolecular Symposia 238(1):16–21. https://doi.org/10.1002/masy.200650603
Hong-Gang L (1995) On the rust removal and anticorrosion of bronzes. World Antiq 04:71–75
Hu X-M, Bi J-H (2007) Study on the bronze cultural relics corrosion and protection. J Anhui Univ (natural Sciences) 031(004):77–80
Jayawardena RS, Purahong W, Zhang W, et al (2018) Biodiversity of fungi on Vitis vinifera L. revealed by traditional and high-resolution culture-independent approaches. Fungal Divers 90:1–84. https://doi.org/10.1007/s13225-018-0398-4
Ji H, Zhang L, Liu J, Wang F (2016) Progress on analytical method for isothiazolinone derivatives used as industrial biocides. Chem Reagents 38(6):523–527. https://doi.org/10.13822/j.cnki.hxsj.2016.06.008
Jing J, Cao Y, Wang J (2017) Detection of Fungicides in Food Contact Paper and Wood Products. Food Science 38(20):256–261
Kathiravan MK, Salake AB, Chothe AS, Dudhe PB, Gadhwe S (2012) The biology and chemistry of antifungal agents: a review. Bioorg Med Chem 20(19):5678–5698. https://doi.org/10.1016/j.bmc.2012.04.045
Leplat J, François A, Bousta F (2020) Parengyodontium album, a frequently reported fungal species in the cultural heritage environment. Fungal Biol Rev 34(3):126-135. https://doi.org/10.1016/j.fbr.2020.06.002.
Leys NMEJ, Ryngaert A, Bastiaens L, Verstraete W, Top EM, Springael D (2004) Occurrence and phylogenetic diversity of Sphingomonas strains in soils contaminated with polycyclic aromatic hydrocarbons. Appl Environ Microbiol 70(4):1944. https://doi.org/10.1128/AEM.70.4.1944-1955.2004
Li Guo-Qing et al.(1984) Study on the equilibrium water content of three kinds of hull wood of Song Dynasty ships in Quanzhou Bay and 14 kinds of modern wood in Quanzhou area. J Fujian Coll for 01:52–62
Li D-F, Lu H, Zhou Y (2010) Research on the antimicrobial effect of catechine, chitooligo saccharide and zinc oxide nanoparticles on freezing-dehydration pre-treated ancient timber. Sciences of Conservation and Archaeology (01):60-64
Li Z, Fu Q, Wang Z, Li T, Zhang H, Guo F, Wang Y, Zhang J and Chen C (2015) TceSR two-component regulatory system of Brucella melitensis 16M is involved in invasion, intracellular survival and regulated cytotoxicity for macrophages. Lett Appl Microbiol 60:565-571. https://doi.org/10.1111/lam.12408
Li N, Han X, Xu S, Li C, Wei X, Liu Y (2018) Glycoside hydrolase family 39 β-xylosidase of Sphingomonas showing salt/ethanol/trypsin tolerance, low-pH/low-temperature activity, and transxylosylation activity. J Agric Food Chem 66. https://doi.org/10.1021/acs.jafc.8b03327.
Li Y, Tang B-L, Ren X-B, Dang Y-R, Sun L-L, Zhang X-Y (2019) Complete genome sequence of Flavobacterium arcticum SM1502T, exhibiting adaption to the Arctic marine salty environment. Mar Genomics 47:100670–100670. https://doi.org/10.1016/j.margen.2019.03.005
Liu Z, Wang Y, Pan X, Ge Q, Ma Q, Li Q (2017) Identification of fungal communities associated with the biodeterioration of waterlogged archeological wood in a Han dynasty tomb in China. Front Microbiol 8:1633. https://doi.org/10.3389/fmicb.2017.01633
Ma W, Wu F, Tian T, He D, Zhang Q, Gu J-D, Duan Y, Ma D, Wang W, Feng H (2020) Fungal diversity and its contribution to the biodeterioration of mural paintings in two 1700-year-old tombs of China. Int Biodeterior Biodegradation 152:104972. https://doi.org/10.1016/j.ibiod.2020.104972.
Masai E, Katayama Y, Nishikawa S, Fukuda M (1999) Characterization of Sphingomonas paucimobilis SYK-6 genes involved in degradation of lignin-related compounds. J Ind Microbiol Biotechnol 23(4–5):364–373. https://doi.org/10.1038/sj.jim.2900747
Mbarki S, Cerdà A, Brestic M, Mahendra R, Abdelly C, Pascual JA (2017) Vineyard compost supplemented with Trichoderma harzianum T78 improve saline soil quality. Land Degradation Dev 28(3):1028–1037. https://doi.org/10.1002/ldr.2554
Meng X, Gong X, Wang Q (2020) Analysis of microbial community structure in urban sewage with CAST process in the upper reaches of the Hanjiang River. J Ankang Univ 032(001):101–105
Nugarli MP; Bartolini M. The protective substances for outside Bronze Works of Art: an evaluation of resistance to biodeterioration. Moropoulou AI, Zezza F, Kollias E, and Papachristodoulou I (Eds) Proceedings: 4th International Symposium on the Conservation of Monuments in the Mediterranean: new concepts, technologies and materials for the conservation of historic cities). Athens: Technical Chamber of Greece. Rhodes (pp. 257–264 (v. 3) 6–11 May 1997
Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO, Amund OO (2008) Pyrene-degradation potentials of Pseudomonas species isolated from polluted tropical soils. World J Microbiol Biotechnol 24(11):2639–2646. https://doi.org/10.1007/s11274-008-9790-7
Pan X, Ge Q, Pan J (2015) Damage to ancient mural paintings and petroglyphs caused by Pseudonocardia sp.-a review. Acta Microbiol Sinica 55(007):813–818
Polo A, Cappitelli F, Brusetti L, Principi P, Villa F, Giacomucci L (2010) Feasibility of removing surface deposits on stone using biological and chemical remediation methods. Microb Ecol 60(1):1–14. https://doi.org/10.1007/s00248-009-9633-6
Rasool A, Irum S (2014a) Toxic metal effect on filamentous fungi isolated from the contaminated soil of Multan and Gujranwala. Journal of Bioresource Management 1(2):38–51. https://doi.org/10.35691/JBM.4102.0006
Rasool A, Irum S (2014b) Toxic effect of heavy metals on filamentous fungi isolated from contaminated soil of Kasur. International Journal of Scientific & Engineering Research, ISSN 2229-5518, Volume 5, Issue 10, 2014
Rong-Yan Z, Shi-Zhen Z, Hong-Lu H (2013) Factors influencing production of siderophore from Pseudomonas c-12. Guizhou Agric Sci 41(01):112–114
Rougeron A, Schuliar G, Leto J, Sitterlé E, Landry D, Bougnoux M-E, Kobi A, Bouchara J-P and Giraud S (2015) The environmental reservoirs of PSC. Environ Microbiol 17:1039–1048. https://doi.org/10.1111/1462-2920.12472
Song M (1992) Relationship between corrosion of metal relics and environment. Journal of National Museum of Chinese History 000:68-73
Sterflinger K, Piñar G (2013) Microbial deterioration of cultural heritage and works of art – tilting at windmills? Appl Microbiol Biotechnol 97:9637–9646. https://doi.org/10.1007/s00253-013-5283-1
Shokralla S, Spal JL, Gibso J, Hajibabae M (2012) Next-generation sequencing technologies for environmental DNA research. Mol Ecol 21(8):1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x
Sultan S, Faisal M (2016) Isolation and characterization of iron and sulfur oxidizing bacteria from coal mines. J Environ Earth Sci 6(3). ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Suphaphimol N, Suwannarach N, Purahong W, Jaikang C, Pengpat K, Semakul N, Yimklan S, Jongjitngam S, Jindasu S, Thiangtham S, Chantawannakul P, Disayathanoowat T (2022) Identification of Microorganisms Dwelling on the 19th Century Lanna Mural Paintings from Northern Thailand Using Culture-Dependent and -Independent Approaches. Biology 11(2):228. https://doi.org/10.3390/biology11020228
Wan J (2019) Disease characteristics and protection methods of marine iron cultural relics. Hakka Cultural 000(004):31–37
Ward DM, Weller R, Bateson MM (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345(6270):63–65. https://doi.org/10.1038/345063a0
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols A Guide Methods Appl 1:315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
Wu J, Zhang Y (2008) The integral salvage of ancient sunken vessel Nanhai I. Navig China 33(12):39–46
Xue X (2015) Microbiology research on N and P removal in mixed wastewater of constructed wetlands. Master's thesis, Tianjin University, Tianjin, China
Yang C, Yue F, Cui Y, Xu Y, Shan Y, Liu B (2018) Biodegradation of lignin by Pseudomonas sp Q18 and the characterization of a novel bacterial DyP-type peroxidase. J Indust Microbiol Biotechnol 45:913–927. https://doi.org/10.1007/s10295-018-2064-y
Yin L, Jia Y, Wang M, Yu H, Jing Y, Hu C, Zhang F, Sun M, Liu Z, Chen Y, Liu J, Pan J (2019) Bacterial and Biodeterioration Analysis of the Waterlogged Wooden Lacquer Plates from the Nanhai No. 1 Shipwreck. Applied Sciences 9(4):653. https://doi.org/10.3390/app9040653
Yoon HS, Aslam Z, Song GC, Kim SW, Jeon CO, Chon TS (2010) Flavobacterium sasangense sp. nov. isolated from a wastewater stream polluted with heavy metals. Int J Syst Evol Microbiologyn 60:267. https://doi.org/10.1099/ijs.0.66923-0
Yu Q-L, Yang Q-Y (2005) Diseases and preservation environment of cultural relics. Relics Museol 000(001):24–27.

Zarasvand KA, Rai VR (2016) Identification of the traditional and non-traditional sulfate-reducing bacteria associated with corroded ship hull[J]. 3 Biotech 6:197
Zhang Xiao-Rong, Hao Xin-Ben (1998) Corrosion of metal cultural relics by microorganisms. Relics Museol 02:91–92
Zhang Yan-Hong, Liu Zi-Jun, Liu Gen-Liang, Li Bin, Pan Jiao, Ma Qing-Lin (2019) Isolation and identification of fungi from some cultural relics and packaging boxes in Tianjin Museum storerooms. Sci Conserv Archaeol 31(02):63–69
Zhang F, Li L, Sun M, Hu C, Zhang Z, Shao H (2019) Fungal community analyses of a pirogue from the Tang Dynasty in the National Maritime Museum of China. Appl Sci 9(19):4129. https://doi.org/10.3390/app9194129
Zhang G, Gong C, Gu J, Katayama Y, Someya T, Gu J-D (2019) Biochemical reactions and mechanisms involved in the biodeterioration of stone world cultural heritage under the tropical climate conditions. Int Biodeterior Biodegradation 143:104723. https://doi.org/10.1016/j.ibiod.2019.104723.
Zhi-Zhen Y, Jing-Juan T, Hong W, Wei-Min T, Hong-Hong G (1989) Determination of efficiency of detergents for dechlorination of bronze. Sci Conserv Archaeol 1(01):34–39

Publisher's note