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Microbial deterioration of tsunami-affected paper-based objects: A case study

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ABSTRACT

Huge numbers of cultural objects were damaged by microorganisms after having been soaked with seawater from the tsunami that followed the Great East Japan Earthquake on March 11, 2011. Among the cultural objects that were damaged, paper-based objects in the tsunami area were severely affected. In the present study, a culture-based analysis and on-site investigations were carried out to determine the extent of the microbial deterioration of paper-based objects (i.e., administrative documents and historical documents). Dark-colored fungi were isolated from black-spotted paper as *Stachybotrys chartarum*, which was observed to grow significantly in mineral salt medium supplemented with cellulose as the sole nutrient source; growth was shown in 10% NaCl. Two isolates from light red-spotted paper were related to *Streptomyces* sp. and *Myxotrichum* sp., respectively. These isolates generated red pigments in the mycelia and on the culture plates. *Penicillium*-related isolates were dominant in the sample tested, and they showed a higher level of NaCl tolerance. From the results of our on-site investigations, a unique discoloration, mainly black- and light red-spotted alterations caused by microbes, was observed on many paper-based objects that had been left wet for several months. The adhesive tape method applied to the black-spotted and red-spotted areas on damaged documents, *Stachybotrys*, *Chaetomium* and *Cladosporium*, and *Actinomycetales* bacterium were observed, respectively. With regard to the discoloration found on tsunami-affected paper-based objects, *Stachybotrys* and *Streptomyces* sp. seem to be responsible for the black- and red-spotted paper alterations, respectively.

1. Introduction

Many historical and cultural important objects are at risk of microbial deterioration, and no material is immune to microbial attack. Microorganisms have been shown to play a role in the deterioration of historic paintings, woods, paper, textiles, waxes, polymers and coatings, glass, metals, and stone (Mitchell and McNamara, 2010). In most cases, the prevention of microbial deterioration aims at reducing the water surrounding the cultural objects, because the availability of water is considered the main factor for microbial activities (Caneva and Ceschin, 2008). However, undesirable water from artificial accidents (e.g., pipe breaks, rain infiltration, air-conditioning system trouble) or unexpected natural disasters (e.g., floods, tsunamis, hurricanes) can enable microorganisms to colonize very rapidly on objects.

When the Great East Japan Earthquake triggered a tsunami in March 2011, a large number of cultural objects and documents were severely damaged at the Pacific coast side of the Tohoku region in Japan. The objects left wet for several months after having been soaked with seawater from the tsunami suffered significant microbial deterioration as well as direct tsunami damage (e.g., loss and breakage). Among the cultural objects damaged by the tsunami, a unique discoloration of paper by microbes (mainly black- and light red-spotted alterations) were commonly observed in tsunami-affected areas. The microbial deterioration of paper generally causes different types of damage, depending on the organisms responsible for the attack. Some paper-degrading fungi and bacteria are capable of dissolving cellulose fibers through the action of cellulolytic enzymes, and they may produce pigments or organic acids which cause serious damage to paper-based objects (Nyuksha, 1983; Ciferri et al., 2000; Michaelsen et al., 2009; Sterflinger and Pinzari, 2012).

The present study aimed to explore the physiological and phylogenetic characteristics of microbes that colonized on the tsunami-affected paper-based objects. Studies on the isolation, identification and characterization of microbes on tsunami- or

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other seawater-affected paper-based objects seem to be rare, and it is expected that the findings from such studies will provide fundamental information about both the initial conservation steps to take after disasters and the potential health risks to the individuals who salvage or restore the damaged objects for future disaster.

2. Materials and methods

2.1. Microbial analysis of deteriorating paper samples

2.1.1. Sample collection

For isolation and microscopic observation, two types of deteriorating paper samples were obtained from tsunami-affected areas in the Tohoku region. The city of Kamaishi situated in the southeastern section of Iwate Prefecture in the Tohoku region, at the Pacific coast, was severely damaged by the earthquake and tsunami in March 2011. A large number of administrative documents at the basement of Kamaishi City Office were left wet for two months after having been soaked with seawater. A paper envelope (the "KEP" sample; abbreviations for microbial strains, Fig. 1b) was collected from the Kamaishi City Office in May 2011. The other is Japanese paper in the underlining of a painted folding screen (the "IJP" sample; abbreviations for microbial strains, Fig. 1a) collected in August 2011. This sample had also been soaked with seawater by the tsunami at a private home in the city of Ishinomaki located in the northeastern section of Miyagi Prefecture in the Tohoku region, at the Pacific coast.

2.1.2. Light microscopic and scanning electron microscopic analysis

For light microscopic observation, the KEP sample was mounted on a slide glass with a drop of lactophenol cotton blue mounting medium. The preparations were sealed with nail polish and observed under a light microscope (BX53, Olympus) as described above. For the scanning electron microscopic (SEM) analysis, a small amount of the KEP sample was examined by SEM (model S-3700N; Hitachi, Tokyo) under low vacuum (LV) conditions inside the sample chamber.

2.1.3. Fungal and bacterial isolation and morphological observations

Fungal and bacterial strains were isolated from deteriorating spots of the KEP and IJP samples by using direct inoculation methods. Each paper sample was placed on half-strength cornmeal agar (CMA; BD, Franklin Lakes, NJ, USA), 8.5 g L^{-1}; agar (BD), 7.5 g L^{-1} and potato dextrose agar (PDA; BD, 39 g L^{-1}). Fungal and bacterial isolates were identified on the basis of cultural microscopic morphology with light microscope and on the basis of their specific gene sequences, as described in the next section.

2.1.4. DNA extraction and PCR conditions of the 18S-26/28S internal transcribed spacer (ITS) region and 16S rRNA gene sequences

Pure cultures of isolates were grown on the half-strength CMA medium for 5–10 days at 25 °C prior to DNA extraction. DNA was extracted using ISOPLANT (Nippon Gene, Toyama, Japan) per the manufacturer's instructions. We assessed the extracted DNA using standard electrophoresis through a 1% (w/v) agarose gel (Solana; Rikaken, Nagoya, Japan) followed by staining with GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA).

Polymerase chain reaction (PCR) amplification was carried out in a reaction mixture containing 100 ng of template DNA, *Ex Taq* buffer (Takara, Otsu, Japan), dNTPs (Takara), *Ex Taq* DNA polymerase (Takara) and each of the two primers. The PCR primers for the fungal 18S-26/28S internal transcribed spacer (ITS) region sequences were ITS1-F (Gardes and Bruns, 1993) primer (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (White et al., 1990) primer (5′-TCCTCCGCTTATTGATATGC-3′), and those for bacterial 16S rRNA gene were 10F primer (5′-AGTTTGATATCCTGGCTCAG -3′, corresponding to positions 10–27 of the *Escherichia coli* 16S rRNA gene) and 1541R primer (5′-AAGGAGGTGATCCAGCCG-3′, positions 1524–1541). PCR was done in a PCR Thermal Cycler Dice (Takara) using the following parameters: for fungi, 4 min at 94 °C followed by 35 cycles each of 35 s at 94 °C, 55 s at 52 °C, and 2 min at 72 °C and final extension at 72 °C for 10 min; for bacteria, 5 min at 95 °C followed by 25 cycles each of 30 s at 95 °C, 1 min at 60 °C, and 1 min at 72 °C.

2.1.5. Sequencing and phylogenetic analysis

We performed the sequencing of PCR products using a DNA sequencer (model 3130x, Applied Biosystems, Foster City, CA) and an ABI PRISM™ Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The determined sequences were analyzed using Molecular Evolutionary Genetics Analysis software (MEGA, version 5.05) (Tamura et al., 2011) and aligned together with reference sequences obtained from the GeneBank, EMBL and DDBJ using the CLUSTAL W program included in MEGA 5.05.

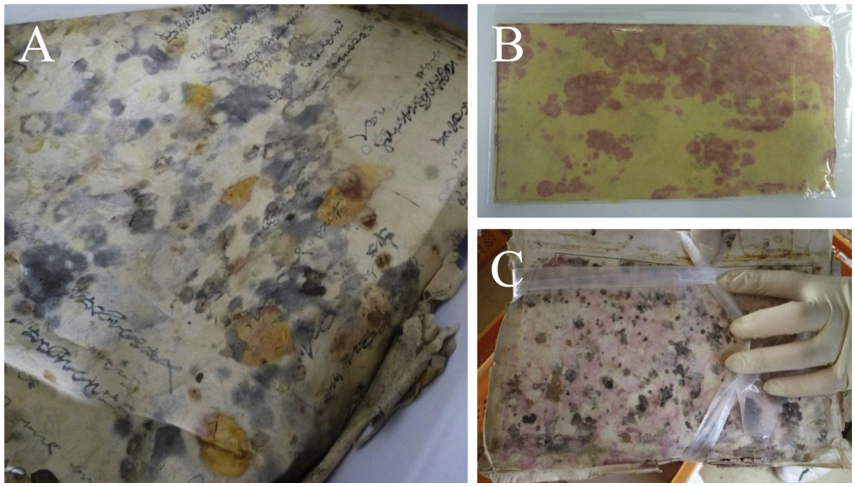

Fig. 1. The Japanese paper in the underlining of a painted folding screen (A) from a private home in the city of Ishinomaki. A envelope paper (B) from the basement of Kamaishi City Office. Black- and light red-spotted alterations on administrative documents (C) from the Kamaishi City Office.