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Isolation of DNA from preserved woods for identification of wood species through genetic analysis regardless of the age of wood production

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[Scope and main objectives]

- Species identification of wood provides important information for archaeology, restoration of cultural assets, preventing illegal logging, and more.
- Wood species are usually identified based on their anatomical features with the use of a microscope.
- However, this method may not be able to distinguish between anatomically similar species or subspecies.
- To overcome this problem, wood species need to be identified at the molecular level using DNA sequencing.
- However, unlike living plant cells, wood is difficult to pulverize using a mortar, and DNA extraction from dried wood is challenging.
- In this study, we propose a pretreatment and pulverization process for stably extracting DNA for species identification from dried wood.

[Innovative approaches]

- DNA from dried wood is expected to dry and stick to the inside of the cell wall ➔ **Hydration** is used as a pretreatment that allows separation and elution with minimal DNA degradation.
- **Powdering with sandpaper** of dried wood for destruction of cells for DNA elution with following advantages
  - No special equipment is required.
  - Anyone can use it at ease.
  - The price is cheap.
  - Less pollution because it is used once
  - The roughness of sandpaper can be adjusted according to the wood.
- Safely confirm DNA elution using **the chloroplast gene**, *rpoB*, with the highest number of genes per cell in plants

[Results]

- **Powdering wood using sandpaper**

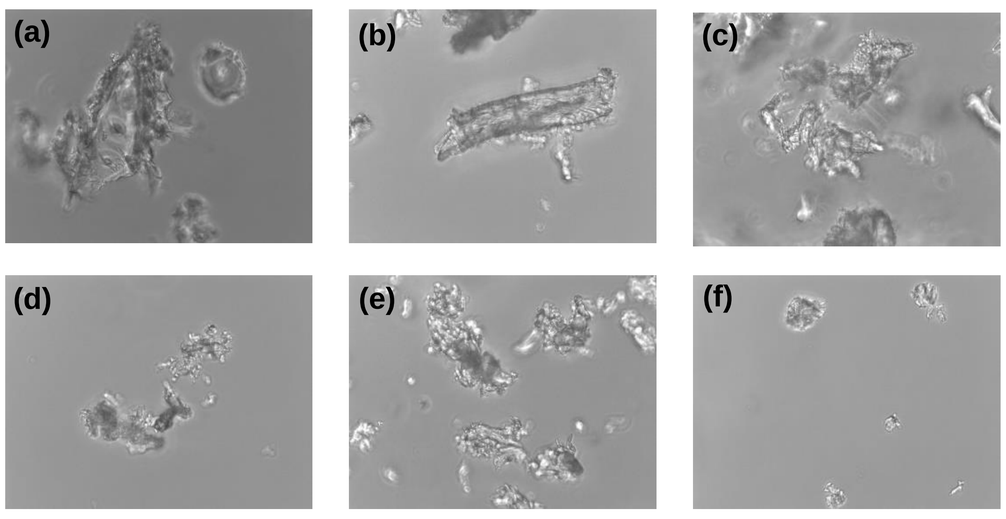

Fig. 1. Pulverizing the wood of *Pinus rigida* using sandpaper. Wood samples were pulverized using sandpaper of different degrees of roughness: 40 grit (a), 50 grit (b), 60 grit (c), 80 grit (d), 100 grit (e), and 220 grit (f). The images of wood particles were obtained using a microscope at 400× magnification. Scale bar: 100 μm.

[Results]

- **Effect of hydration time on DNA extraction**

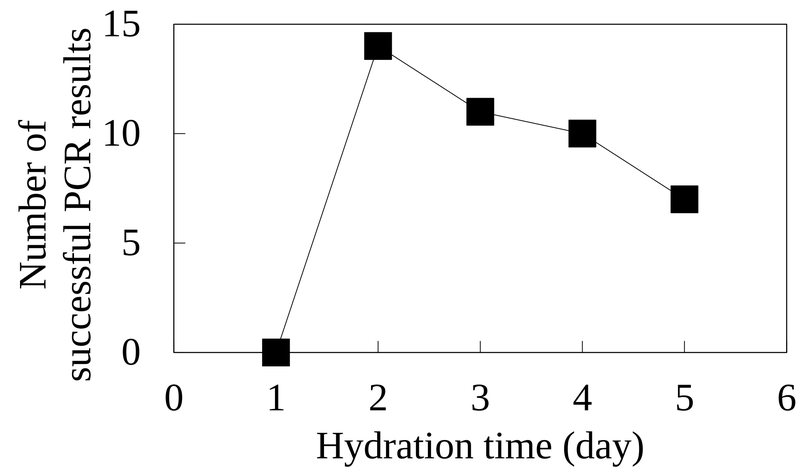

Fig. 2. Effect of hydration time of wood powder on polymerase chain reaction (PCR). The number of successful PCRs of the *rpoB* gene (Y axis) is shown as a function of the DNA extracted from wood powder hydrated for 1 to 5 days (X axis). Eighteen independent hydration experiments were conducted.

- **Identification of SNPs of *rpoB* in *P. rigida***

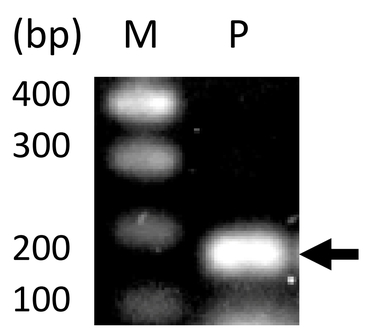

Fig. 3. Polymerase chain reaction (PCR) amplification of the *rpoB* gene from DNA extracted using the method developed in this study. The amplified *rpoB* gene was separated by gel electrophoresis using 1.5% agarose. The arrow on the right side indicates the amplified *rpoB* gene fragment in lane P. M: marker.

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                                  **
(THIS WORK)   (1) TTTCTCCTAC ATCGATCTCT AATTTCGATC TTCTTCCCCA (40)
 KX255674.1   (1) TTTCTCCTAC ATCGATCTCT AATTTCGATC TTCTTCCCCA (40)
 KC427273.1   (1) TTTCTCTCAC ATCGATCTCT AATTTCGATC TTCTTCCCCA (40)
 KR476379.1   (1) TTTCTCCCAC ATCGATCTCT AATTTCGATC TTCTTCCCCA (40)
 JN854213.1   (1) TTTCTCCCAC ATCGATCTCT AATTTCGATC TTCTTCCCCA (40)
 KR873010.1   (1) TTTCTCCCAC ATCGATCTCT AATTTCGATC TTCTTCCCCA (40)

                                       *                    **
(THIS WORK)  (41) ATCTGAAATC AAAGTGCCAG TATATATATA ATTTATTCTA (80)
 KX255674.1  (41) ATCTGAAATC AAAGTGCCAG TATATATATA ATTTATTCTA (80)
 KC427273.1  (41) ATCTGAAATC AGAGTGCCAG TATATATATA ATGAATTCTA (80)
 KR476379.1  (41) ATCTGAAATC AGAGTGCCAG TATATATATA ATTAATTCTA (80)
 JN854213.1  (41) ATCTGAAATC AGAGTGCCAG TATATATATA ATTTATTCTA (80)
 KR873010.1  (41) ATCTGAAATC AAAGTGCCAG TATATATATA ATTTATTCTA (80)

                                       *
(THIS WORK)  (81) TTATGGTCTA ATTCTGAACG GTAATAAATA CCAGGACTTA(120)
 KX255674.1  (81) TTATGGTCTA ATTCTGAACG GTAATAAATA CCAGGACTTA(120)
 KC427273.1  (81) TTATGGTCTA ATTCTGAACG GTAATAAATA CCAGGACTTA(120)
 KR476379.1  (81) TTATGGTCTA ATTCCGAACG GTAATAAATA CCAGGACTTA(120)
 JN854213.1  (81) TTATGGTCTA ATTCTGAACG GTAATAAATA CCAGGACTTA(120)
 KR873010.1  (81) TTATGGTCTA ATTCTGAACG GTAATAAATA CCAGGACTTA(120)
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Fig. 4. Multiple sequence alignment of the *rpoB* gene of *P. rigida* obtained in this study with *rpoB* sequences of the genus *Pinus*. Nucleotides in bold with asterisks above the alignment indicate single nucleotide polymorphisms (SNPs).

[Conclusions]

- In this study, we propose a pretreatment method for wood samples that involves pulverizing the wood samples using 60-grit sandpaper followed by hydration with water for 2 days for DNA extraction.
- DNA isolated by this method was a good template to amplify the *rpoB* gene.
- The method proposed in this study stably eluted DNA from wood and will provides a method for species identification using DNA analysis of wood.

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