

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 4877.15-2019

Gene barcode screening methods - Part 15: Phytophthora

基因条形码筛查方法 第15部分：检疫性叶点霉

(English Translation)

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Foreword

This section is drafted in accordance with the rules given in GB/T 1.1-2009.

This section is proposed and managed by the General Administration of Customs of the People's Republic of China.

This section was drafted. Ningbo Institute of Inspection and Quarantine Science and Technology, Ningbo Customs, People's Republic of China, Microbiology Research Institute, Chinese Academy of Sciences Institute, China Academy of Inspection and Quarantine.

1 Scope

This section specifies the quarantine leaf spot DNA screening method.

This section applies to screening of quarantine leaf spot molds in imported plants and their products.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this article Pieces. For undated references, the latest version (including all amendments) applies to this document.

SN/T 2589 Specification for detection of plant pathogenic fungi

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 Phytophthora fungi

spearieres), Dhodocomycetes, Ascomycota.

A voucher specimen is a long-term preservation that provides a physical basis for identification or research and has sufficient archived information (collection and identification information, etc.) the specimen, it can be a complete biological individual or a part of it, or it can be the genetic material of the organism. The preservation of voucher specimens needs to be detailed Detailed specimen number, storage location and other detailed information for future verification.

The DNA barcode refers to a species that can represent the species in the body, a standard, sufficiently mutated, easily amplified, and relatively short DNA Fragment. Researchers use DNA barcode technology to identify species and inter-species relationships, and modify existing taxonomic conclusions.

The DNA barcode used to identify the quarantine Phytophthora is as follows.

Ribosomal Gene Transcription Spacer (Internal Tracance Striper, ITS for short)

4 Method principle

According to the sequence characteristics of ribosomal transcribed spacer (ITS) of Phyllostachys sp Carry out species screening to determine whether the target fungus is quarantine leaf spot mold and its species classification.

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5.1 Apparatus and equipment

Microscope, autoclave, ultra-clean workbench, tissue breaker, thermostatic water bath, desktop refrigerated centrifuge, micro-spectrophotometer, electric Sub-balances, ultra-pure water meters, vortex shakers, refrigerators, micropipettes (0.5 µL, 2.5 µL, 10 µL, 20 µL,.200 µL, 1000 µL), conventional PCR instruments, nucleic acid electrophoresis instruments, gel

imaging systems, DNA Extraction instrument.

5.2 Main reagents

Purchase the following reagents or the corresponding fungal genome extraction kit. ethylenediamine tetraacetic acid sodium salt ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$), hydrogen NaOH, Tris base, concentrated hydrochloric acid (HCl), cetyltrimethylammonium bromide (CTAB), sodium chloride (NaCl), polyvinylpyrrolidone (PVP), glacial acetic acid (CH_3COOH), distilled water, chloroform (CHCl_3), isoamyl alcohol, 70% ethanol.

Reagents required for PCR and electrophoresis detection. 10 x PCR buffer, deoxyribonucleoside triphosphates (dNTPs), ExTaq polymerase, primer Materials, ultrapure water, DL2000 Marker, agarose, GelRed nucleic acid gel dye.

6.1 Collection and processing of samples

The collection, isolation, purification and cultivation of samples were performed in accordance with the quarantine and identification methods for plant pathogenic fungi of Sn/T 2589.

6.2 Preparation of nucleic acids

Genomic extraction kits are used to prepare DNA, or modified CTAB extraction methods are used, see Appendix A.

6.3 Determination of DNA purity and concentration

The purity and concentration of DNA were measured with a micro-ultraviolet spectrophotometer, and the absorption values at 260 nm and 280 nm were obtained, and the nucleus was calculated.

The purity and concentration of the acid are calculated as follows.

DNA purity = $\text{OD}_{260}/\text{OD}_{280}$ the concentration of DNA = $50 \times \text{OD}_{260} \mu\text{g/mL}$ PCR-grade DNA solution should have an $\text{OD}_{260}/\text{OD}_{280}$ ratio of 1.7 to 1.9.

6.4 Sequence Amplification and Sequencing

Amplify and sequence the ITS gene using universal primers (see Appendix B for specific steps), and place the sequence in the Genbank database or the Chinese inspection Alignment in the pest barcode DNA barcode database, if the sequence similarity with the *Phyllostachys* sp. ITS gene in the database is greater than 98%, then the sequence homology was analyzed.

7 Result judgment

The length of the ITS gene sequence is greater than 500 bp, and it is carried out in the NCBI database or China 's quarantine pest barcode database.

Alignment analysis, if the homology with the *Phyllostachys* sp. ITS gene sequence (see Appendix C) in the database is greater than 98%, the bacteria is determined to be a leaf point Mold.

If the determined sequence is 100% homologous to the reference sequence of the quarantine leaf spot mold of Appendix D, the bacteria is determined to be grape rot fungus.

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8.1 Sample preservation

Strains isolated and eventually identified as quarantine target fungi should be transferred to the test tube slope, and registered and signed at 4 °C Keep it in a low temperature refrigerator, and transfer it regularly (30d ~ 60d), and freeze it for a long time if necessary.

8.2 Records of results and data retention

Complete experiment records include. sample source, type, time, experiment time, place, method and result, etc.

Sign with the reviewer. Photographs of electrophoresis results are required for PCR gel electrophoresis detection, and sequences need to be saved in electronic files.

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Appendix A

(Informative appendix) DNA extraction method A. 1 Take an appropriate amount of mycelia (500mg) and sterilized glass beads in a 2mL centrifuge tube, add 500μL 2 x CATA (60 ° C), and place the tissue cell wall on a tissue crusher. Incubate in a water bath for 30-60 minutes, occasionally shaking and mixing uniform;

A. 2 Add 500 μL (1. 1, equal volume) of chloroform/isoamyl alcohol (24. 1), mix well (3 min), 2,000 rpm, and centrifuge for 15 min; A. 3 Extract the supernatant, add 250 μL (1. 1, equal volume) of chloroform/isoamyl alcohol (24. 1), 2,000 rpm, and centrifuge for 15 min; repeat this step once;

A. 4 extract the supernatant, add an equal volume of isopropanol, mix well and precipitate for 30 min;

A. 5 After precipitation, centrifuge at 12,000 rpm for 10 min;

A. 6 Discard the liquid phase, add 400 μL of 70% ethanol, wash with shaking, and then