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Detection and Identification of *Xylophilus Ampelinus* (Panagopoulos) Willems *et al.*

葡萄细菌性疫病菌检疫鉴定方法

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Detection and Identification of *Xylophilus Ampelinus* (Panagopoulos) Willems *et al.*

1 Scope

This Standard specifies the detection and identification methods (separation culture, immunology and molecular biology, etc.) for *xylophilus ampelinus* (panagopoulos) Willems *et al.*

This Standard is applicable to the detection and identification of *xylophilus ampelinus* (panagopoulos) Willems *et al.* in grapes and the propagating materials.

2 Normative References

The content of the following documents constitutes indispensable clauses of this document through normative references in the text. In terms of references with a specified date, only versions with a specified date are applicable to this document. In terms of references without a specified date, the latest version (including all the modifications) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

SN/T 2122 Sampling Methods for the Quarantine of Plants and Their Products for Import and Export

3 Terms and Definitions

There are no terms and definitions that need to be defined in this document.

4 Classification Information of *Xylophilus Ampelinus* (Panagopoulos) Willems *et al.*

Chinese name: 葡萄细菌性疫病菌、葡萄嗜木质菌

Scientific name: *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*,1987

Synonym: *Xanthomonas ampelina* Panagopoulos,1969

English name: canker of grapevine, bacterial blight of grapevine

Taxonomic status: Bacteria, Eubacteria, Proteobacteria, Betaproteobacteria,

Burkholderiales, Comamonadaceae, *Xylophilus*

See other information in Appendix A.

5 Method Principle

In accordance with the specific reaction between *Xylophilus ampelinus* (Panagopoulos) Willems *et al.* and the antibody, conduct immunological detection on the test object. In accordance with the specific DNA sequence of *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*, conduct molecular biological detection. In accordance with the cultural characteristics, biological characteristics and hazard symptoms of *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*, conduct separation culture on the pathogenic bacteria, if necessary, conduct pathogenicity detection.

6 Reagents, Materials and Culture Medium

6.1 Reagents and Materials

Unless it is otherwise specified, all reagents used for the tests are analytically pure or biochemical reagents.

Reagents: 75% alcohol, glucose, agarose, sterile double distilled water, sodium dodecyl sulfate (SDS), chloroform, isoamyl alcohol, phenol, isopropanol, absolute ethanol, proteinase K, nucleic acid dye, DNA molecular mass reference substance, 50 × TAE electrophoresis buffer.

See Appendix B for information on the reagents and their preparation.

Materials: plant genomic extraction kit, bacterial genomic extraction kit, common PCR reaction kit and fluorescent PCR reaction kit, etc.

6.2 Culture Medium

See Appendix C for information on YPGA culture medium, nutrient agar medium (NA), culture medium and its preparation. YPGA is recommended for the separation culture medium, and NA may also be used as a substitute.

7 Instruments and Utensils

7.1 Instruments

Biological microscope (magnification by more than 400 times), constant-temperature incubator, electronic balance (one thousandth), plant light incubator, refrigerator, centrifuge, microplate reader, electrophoresis instrument, PCR amplification instrument and real-time fluorescence quantitative PCR instrument, etc.

molecular biological detection. In accordance with laboratory conditions, one of the following methods (8.4.2 or 8.4.3) may be selected for the detection.

8.4.2 Common PCR and nested PCR

DNA extracted from the extract or other separated objects is used as a template for the common PCR and nested PCR detection. The detection steps and conditions shall comply with D.2.

8.4.3 Real-time florescence PCR

DNA extracted from the extract or other separated objects is used as a template for the real-time fluorescence PCR detection. The detection steps and conditions shall comply with D.3.

8.5 Separation of Pathogenic Bacteria

8.5.1 Plant materials with symptoms

Take the liquid obtained in 8.2.1 and directly apply it to the YPGA plate; apply 5 ~ 10 petri dishes for each sample. At 25 °C, cultivate them; from the third day, observe the growth of colonies on the plate every day. Typical colony, which grows slowly on the culture medium, is smooth and non-sticky, shiny, slightly convex, light yellow, circular and with regular edges. After 7 d ~ 12 d of culture, the size is about 2 mm. If necessary, conduct purification culture once on the colony.

8.5.2 Plant materials without symptoms

Take 1.0 mL of the liquid obtained in 8.2.2, use sterile water or phosphate buffer solution to dilute it by 10 times, 100 times and 1,000 times. Respectively take 200 μL of the diluent for coating separation on the YPGA plate. For each dilution degree, apply 5 ~ 10 petri dishes. The cultivation method is the same as 8.5.1.

8.6 Pathogenicity Detection

If necessary, pathogenicity determination may be carried out.

The pathogenicity experiments are carried out on potted susceptible vine stems or new cutting seedlings. Collect the suspected bacteria freshly cultured on YPGA culture medium and use sterile water to prepare a bacterial suspension of about 1×10^8 CFU/mL. On the seedling cane, use a sterile knife to cut a fresh wound of 2 cm ~ 4 cm long, which reaches the depth of the vascular bundle. Inoculate 1 drop ~ 2 drops of the bacterial suspension to the wound or fresh wound of the leaf; use sterile cotton moistened by sterile water to cover the wound and use tin foil sheet to wrap it; repeat for 5 ~ 10 times. In the same way, use the standard strain of *xylophilus ampelinus* (panagopoulos) Willems *et al.* as a positive control; use sterile water as a negative control; repeat for at least 2 times. Place the inoculated grape seedlings at 20 °C ~

27 °C; the sunshine duration is 14 h ~ 18 h; cultivate for 3 ~ 4 weeks in an environment with sufficient moisture and fertilizer.

The pathogenicity experiments may also be carried out on the susceptible vine stems that have rooted in the test tube. Collect the suspected bacteria freshly cultured on YPGA culture medium and use sterile water to prepare a bacterial suspension of about 1×10^9 CFU/mL. Use a sterile knife to cut off the topmost part of the vine stem; inoculate 1 drop of the bacterial suspension onto the wound; use sterile cotton moistened by sterile water to cover the wound and use tin foil sheet to wrap it; repeat for 5 ~ 10 times. In the same way, use the standard strain of *xylophilus ampelinus* (panagopoulos) Willems *et al.* as a positive control; use sterile water as a negative control; repeat for at least 2 times. Place the inoculated grape seedlings at 20 °C ~ 27 °C; the sunshine duration is 14 h ~ 18 h; cultivate for 3 ~ 4 weeks in a humid environment.

9 Result Determination

9.1 Plant Tissues with Typical Symptoms

In accordance with 8.3 and 8.4, test the sample extracts or suspected strains. If the test results of both methods are positive, then, it can be determined that *xylophilus ampelinus* (panagopoulos) Willems *et al.* is detected. If the test results of both methods are negative, then, it can be determined that *xylophilus ampelinus* (panagopoulos) Willems *et al.* is not detected. If only one of the test results is positive, conduct separation of pathogenic bacteria to obtain typical or suspected strains, which are tested through one of the methods in 8.3 or 8.4 (a different method than the initial screening test that obtains positive result). If the result is positive, then, it can be determined that *xylophilus ampelinus* (panagopoulos) Willems *et al.* is detected. If typical or suspected strains are not separated, or the strain identification result is negative, then, it can be determined that *xylophilus ampelinus* (panagopoulos) Willems *et al.* is not detected.

9.2 Plant Tissues without Typical Symptoms

Adopt the methods in 8.3 and 8.4 to conduct detection and screening of the sample extracts. In the screening results, if there is 1 positive result or both are positive results, then, conduct separation of pathogenic bacteria to obtain typical or suspected bacterial colonies, which are tested through 8.3 and 8.4. If necessary, in combination of the results of the pathogenicity experiments, if there are 2 positive results, then, it can be determined that *xylophilus ampelinus* (panagopoulos) Willems *et al.* is detected; if there are 2 negative results, then, it can be determined that *xylophilus ampelinus* (panagopoulos) Willems *et al.* is not detected; if there is 1 positive result, then, the PCR method in 8.4 that is different from the method that obtains positive result or the pathogenicity experiments may be adopted for further identification. If the result of the further identification is negative, then, it can be determined that *xylophilus ampelinus*

Appendix A

(informative)

Xylophilus Ampelinus* (Panagopoulos) Willems *et al.

A.1 Distribution

This pathogen is currently distributed in: South Africa, Tunisia, Japan, Turkey, Austria, Belgium, Bulgaria, France, Greece, Italy, Moldova, Netherlands, Portugal, Serbia, Slovenia, Spain, Switzerland, United Kingdom (UK), Argentina and Uruguay.

A.2 Host

Natural host: grape (*Vitis vinifera Linn.*) is currently the only known host.

A.3 Onset Characteristics

From spring to June each year, the symptoms can be observed on the vines. On 12 cm ~ 30 cm long rattan canes, the lower 2 or 3 segments are often the first to be attacked, which is then gradually expanded upward. In the early stage, reddish-brown streaks appear, extending from the stem of the cane to the top, and gradually develop into lenticular cracks or ulcers, sometimes reaching deep into the pith, and the cane is finally wilted and withered (see Figure A.1). On some young branches, there is less discoloration; the whole branch is withered and the diseased branch is shorter, which leads dwarf phenomenon of the grapes. On the cross section of the stem, the tissue is brown. The symptoms of the diseased main branches and branches are the same as the symptoms generated by the tip of the branches. On the leaves, pathogens invade the leaves through the petiole and vascular bundles, and the leaves are often withered (see Figure A.2). In addition, the pathogens directly infect the leaves through the stomata. This type of infection causes reddish-brown horn spots on the leaves. When the pathogens infect from the stomata, it turns reddish-brown. When the temperature is relatively high, yellowish bacterial ooze can be seen flowing out of the diseased leaves. After the flowers are infected, they turn black and wither before maturity. The pathogens can also infect the roots of the grapes, whether it is grafted plants or plants on their own rootstocks, they will decelerate the growth of the tip of the branches. The life cycle of *xylophilus ampelinus* (panagopoulos) Willems *et al.* has not been thoroughly elucidated yet, and the initial infection mainly occurs on 1 ~ 2 year-old branches, and invades the plants through leaves, flowers and fruits. The pathogens propagate with plants, especially in humid and windy weather, which makes it easier for the propagation. Then, in early summer, they spread to other new buds. The occurrence of the disease requires warm and humid conditions.

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