

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

SN/T 2622-2019

Replacing SN/T 2622-2010

**Detection and identification of *Xanthomonas axonopodis*
pv.citri**

柑桔溃疡病菌检疫鉴定方法

(English Translation)

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Foreword

This standard is prepared in accordance with the rules given in GB/T 1.1-2009.

This standard replaces Sn/T 2622-2010 "Citrus quarantine identification method", the main changes compared with Sn/T 2622-2010 as follows.

--- Revised the original standard quarantine identification and determination procedures for citrus canker bacteria;

--- Added pictures of citrus canker bacteria;

--- Added more alternative PCR methods such as the real-time fluorescence PCR detection method for citrus canker bacteria;

--- Added the response of citrus ulcer pathogen determination.

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

This standard was drafted. Chengdu Customs of the People's Republic of China, Shanghai Customs of the People's Republic of China.

1 Scope

This standard specifies the quarantine and identification methods for citrus canker bacteria in entry and exit plant quarantine.

This standard applies to the quarantine and identification of citrus canker bacteria in Rutaceae plants and their fruits.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

GB/T 6682 Laboratory water specifications and test methods Sn/t 1157 quarantine regulations for entry and exit plant nursery stock Sn/t 2122 quarantine sampling

3 Basic information on germs

Proteobacteria, gamma-proteobacteria Gammaproteobacteria, Xanthomonadales, Xanthomonaceae

4 Method principle

Harmful symptoms, colony morphological characteristics, pathogenic response, and PCR-specific response of the pathogen are the main basis for its quarantine identification.

5 Major reagents

Unless otherwise specified, the reagents in this standard are analytical reagents or biochemical reagents. For the test water, refer to GB/T 6682.

70% ethanol, peptone, beef extract, yeast extract, sucrose, agar, ethidium bromide (EB), agarose, DNA Marker, TaqDNA polymerase, PCR system, MgCl₂, dNTP, Taqman probe, etc.

6 Equipment

Stereo microscope, super earring bench, autoclave, biological incubator, electronic balance, ordinary refrigerator, ultra-low temperature refrigerator, centrifuge, vortex Mixer, pure water meter, turbidimeter, PCR meter, real-time fluorescence PCR meter, electrophoresis meter, gel imaging system, micro-adjustable sampler, scissors, culture Petri dishes, triangle bottles.

7.1 Quarantine and identification process

The identification process of citrus canker is shown in Figure 1.

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Figure 1 Identification process of citrus canker

7.2 Symptom check

Perform on-site quarantine and take samples according to the procedures specified in Sn/T 1157 and Sn/T 2122 Laboratory symptoms Check to see if the sample to be tested has suspected symptoms (see Appendix A). If there are symptoms, cut out the tissues under the symptoms and observe whether under the microscope There is a spraying phenomenon. If there is a spraying bacteria, isolate the bacteria directly from the spraying tissue.

If the sample is asymptomatic or no symptom is found in the sample, perform a preliminary PCR test. See Appendix B for the PCR method. Samples with positive PCR results Isolation of germs.

7.3 PCR detection

Take asymptomatic or symptomatic samples without spraying bacteria, soak in sterile normal saline at room temperature for 30min or 4 °C refrigerator overnight, shake, and take the soaking solution was centrifuged at 10,000 r/min for 10 min, and DNA was extracted by precipitation. Routine PCR or real-time fluorescence PCR detection (Appendix B) Duck ulcer bacteria DNA was used as a positive control, non-citrus ulcer bacteria DNA was used as a negative control, and double distilled water was used as a blank control.

7.4 Isolation of germs

A 2mm x 2mm piece of tissue was taken from the symptom of the symptomatic sample, and the incision was observed under the microscope at 10 x ~ 20 x times after making the slide Cloudy bacteria spewed from the incision. Cut 5 to 10 small pieces of tissue from each sample to observe spraying bacteria, and isolate 3 to 5 small spraying bacteria.

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Block tissue, such as less than 3 sprayed tissue, can increase the number of microscopic examination of tissue. If spraying bacteria, remove the sprayed tissue pieces and rinse them with sterile water.

70% alcohol surface disinfection treatment for 30s to 60s, rinsed with sterile water three times, transferred to 100μL of sterile water and soaked for 15min; the treatment solution was separated on the NA plate, and 3 to 5 plates were marked for each treatment; Observe

whether there are typical colonies after incubating for 2 to 4 days. Suspect After the colonies were purified three times, the next identification test was performed.

If there is no evidence of bacterial spraying in the sample, take the tissue scissors of the symptom to cut into 2mm square pieces, and add appropriate amount of sterilized water to soak at room temperature.

Soak in 30min or 4 °C refrigerator overnight, take half of the treatment solution at 1000r/min and centrifuge for 10min, precipitate and extract DNA, conventional PCR or real-time Preliminary fluorescence PCR test. The positive sample treatment solution was diluted 10 x, 100 x, and 1000 x, and 100 µL of NA plate was taken, and 5 to 10 plates were coated for each treatment. The cultivation and observation methods are the same as above. If typical colonies are not isolated from PCR-positive samples, increase sample repetition.

Or apply the treatment solution directly to the plate without dilution and observe the culture.

For the initial examination and isolation of asymptomatic samples, refer to this method.

7.5.1 Colony morphological characteristics

The colonies on the NA medium are smooth, opaque, shiny, round, raised, and the edges are intact; it is initially white and then turns light yellow; NBZ the colonies on the medium were light yellow, round, raised, and slime-like; the colonies on the PSA medium were pale yellow, mucus-like, and shiny. Pick Suspicious Bacteria After falling on the NA medium plate and purifying three times, it was subjected to biolug measurement or PCR identification.

See Appendix A for the physiological and biochemical characteristics of the pathogen.

7.5.2 PCR amplification

Suspect isolates were cultured on a NA plate for 48 h at 28 ° C. The bacterial cells were collected to extract DNA for routine PCR or real-time fluorescence PCR detection.

Testing, see Appendix B for testing procedures.

7.5.3 Pathogenicity determination

The suspected isolate was cultured at 28 ° C for 48 hours on NA, and then prepared with 108 CFU/mL bacteria suspension with sterile water.

Grapefruit, Mexican lemon, citrus and sweet orange) young leaves, with sterile water as a negative control. The plant was grown at 28 ° C, and the leaves were observed after 7 days.

Incidence. The pathogens were re-isolated from the tissues where the symptoms appeared, and compared with the original isolates to complete the pathogenicity test.