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

SN/T 5204-2020

Plant pathogenic bacteria screening MALDI-TOF MS method

植物病原细菌筛查 MALDI-TOF MS法

(English Translation)

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Foreword

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

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

Drafting organizations of this document. Shenzhen Customs of the People's Republic of China, Shenzhen Institute of Inspection and Quarantine.

1 Scope

This document specifies the detection method of phytopathogenic bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

This document is suitable for rapid screening and testing of pure cultures of plant

pathogenic bacteria.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

4 Principles of the method

Bacterial cells contain about 200 to 6,000 protein molecules, which account for about 50% of the dry weight of the bacteria, and have species characteristics.

The molecular weight and structure of small peptides can be determined by MALDI-TOF MS to realize the identification of bacterial species.

5.1 Apparatus

Autoclave, light incubator, balance (sensitivity 1/100; 1/10000), water bath, pure water meter, vortex oscillator, desktop Centrifuge, refrigerator, ultra-clean workbench, matrix-assisted laser desorption ionization time-of-flight mass spectrometer, ultrasonic vibrator.

5.2 Utensils

Petri dishes, graduated cylinders, pipettes, alcohol lamps, filter paper, micropipettes (0.5 μL , 2 μL , 10 μL , 20 μL , 100 μL , 200 μL , 1 000 μL), PCR reaction tube (0.2 mL, 0.5 mL, 1 mL), Tip head (0.1 μL ~10 μL , 5 μL ~200 μL , 100 μL ~1000 μL), Mass spectrometry analysis of the sample target.

6 Reagents, solutions and media

See Appendix A for all reagents, solutions and medium formulations.

7.1 Strain culture

The test strains were streaked inoculated into NBY and other related media for activation and culture, and then incubated in the dark at $28\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 48 h before use.

7.2 Bacteria treatment

See Appendix B for specific steps.

7.3.1 Instrument conditions

7.3.1.1

Instrument. Matrix-assisted laser desorption ionization time-of-flight mass spectrometer.

7.3.1.2

Laser energy. According to the sample, it can be adjusted from 0% to 100%.

7.3.1.3

Mass spectrum scanning range. 1000 Da~20000 Da.

7.3.1.4

Detection method. linear positive ion detection method.

7.3.1.5

Microbial fingerprint library. the instrument's own database or self-built equivalent type database.

7.3.2 Calibration of quality control standards

It is recommended to use a mixture of Peptide Calibration Standard and Protein Calibration Standard I before testing each sample.

The mass spectrometer is calibrated with the characteristic peaks of liquid or equivalent standards.

7.3.3 Spot

7.3.3.1

Spot 1 μL of supernatant on the mass spectrometer target, 4 replicates for each sample spot.

7.3.3.2

Take 1 μL of standard calibration solution as a control.

7.3.3.3

After natural drying at room temperature, spot 1 μL of substrate as soon as possible and let it dry.

7.3.4 Identification

Put the sample target board into the instrument, set parameters such as laser energy and mass spectrum scan range, and collect mass spectrum data. And then by the processed mass spectrum data is automatically/manually imported into the local microbial spectrum library, and the obtained mass spectrum analysis spectrum and microbial fingerprint

spectrum library are imported into the library.

Line comparison, the log (score) value is calculated through the instrument's own software, and the type of unknown bacteria is judged.

8 Result judgment

8.1

If the log (score) value is greater than or equal to 2.2 and less than or equal to 3.0, the species of plant pathogenic bacteria can be determined.

8.2

If the log (score) value is greater than or equal to 2.0 and less than 2.2, the genus of the plant pathogenic bacteria can be determined.

8.3

If the log (score) value is less than 2.0, the genus of the plant pathogenic bacteria cannot be determined.

8.4

For further judgement, identification can be carried out according to the relevant standard methods of bacteria; if there is no corresponding standard, identification should be carried out according to conventional bacterial identification methods.

9.1 Sample storage

The stored samples should be stored according to the state of the samples and should be properly stored for 6 months for re-inspection, if trade disputes are involved it should be saved until the dispute is resolved.

9.2 Save the original data

After the sample is tested, the original record sheet, spectrum and inspection report or certificate shall be filed and kept in a safe place for re-inspection, negotiation and arbitration.

9.3 Strain preservation

The isolated strains are stored in ultra-low temperature or freeze-dried for re-inspection, negotiation and arbitration.

Appendix A

(Normative appendix) Reagents, solutions and media A.1 Reagents Anhydrous ethanol, isopropanol, acetonitrile, trifluoroacetic acid (TFA) and methanol, the above organic