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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 5228.6-2019

Rapid screening method for pathogenic microorganisms in exported foods MALDI-TOF MS method Part 6: Bacillus cereus

出口食品中病原微生物快速筛选方法 MALDI-TOF MS法 第6部分：蜡样芽胞杆菌

(English Translation)

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Foreword

SN/T 5228-2019 "The MALDI-TOF MS Method for Rapid Screening of Pathogenic Microorganisms in Exported Foods" is divided into 9 parts.

--Part 1: Vibrio alginolyticus;

--Part 2: Clostridium perfringens;

--Part 3: Staphylococcus aureus;

--Part 4: Cronobacterium;

--Part 5: Vibrio vulnificus;

--Part 6: Bacillus cereus;

--Part 7: Campylobacter jejuni;

--Part 8: Klebsiella pneumoniae;

--Part 9: Pseudomonas aeruginosa.

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This part is part 6 of SN/T 5228-2019.

This part was drafted in accordance with the rules given in GB/T 1.1-2009.

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

Drafting organizations of this section. Chinese Academy of Inspection and Quarantine, Jilin Customs of the People's Republic of China, Yining of the People's Republic of China Customs, Beijing Customs of the People's Republic of China.

1 Scope

This part of SN/T 5228 specifies the MALDI-TOF MS rapid screening method for Bacillus cereus in exported food.

This section applies to the rapid screening of Bacillus cereus in export food.

2 Normative references

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

GB 4789.14 National Food Safety Standard Food Microbiological Inspection Inspection of Bacillus cereus GB/T 6682 Analytical laboratory water specifications and test methods GB 19489 Laboratory Biosafety General Requirements

3 Abbreviations

The following abbreviations apply to this document.

BTS. bacterial test standard.

CHCA. alpha-cyano-4-hydroxy-cinnamic acid (alpha-cyano-4-hydroxy-cinnamic acid).

MALDI-TOF MS. matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry).

4 Biosecurity measures

In order to protect the safety of laboratory personnel, qualified personnel should detect Bacillus cereus, all cultures and waste Objects should be implemented with reference to the relevant regulations in GB 19489.

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5 Method summary

MALDI-TOF MS is a technology applied to the rapid detection of microbial whole cells, mainly based on the fingerprint analysis of microbial characteristic protein.

Complete the identification and classification of microorganisms. Place the sample of the colony to be identified and an appropriate amount of matrix solution on the sample plate, and form after the solvent volatilizes the co-crystallization of the sample and the matrix, the laser is used as the energy source to radiate the co-crystal, the matrix molecules absorb energy and desorb the sample and make the sample Product ionization, after the time-of-flight analyzer, separates the ions of different mass-to-charge ratios to form a microbial-specific mass spectrum. Will be tested Comparing the substance spectrum with the standard protein fingerprint database of known microorganisms, the species of microorganisms can be determined, and the species of microorganisms can be determined.

?identification.

6 Reagents and materials

Except for special instructions, all reagents are chromatographically pure reagents. The experimental water meets the requirements of first-grade water in GB/T 6682.

6.1
Brain Heart Infusion Agar (BHIA).

6.2
Acetonitrile (acetonitrile, ACN).

6.3
Absolute ethanol.

6.4
Trifluoroacetic acid (TFA).

6.5
alpha-cyano-4-hydroxycinnamic acid (CHCA).

6.6
Formic acid.

6.7
BTS standard product.

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6.8
Solvent I. prepared according to the ratio of water. acetonitrile (6.2). trifluoroacetic acid (6.5) to 50.47.5.2.5 (volume ratio).

Now use.

6.9
Preparation of BTS standard solution. Dissolve BTS standard (6.7) with 50 µL solvent I (6.8). Pipette repeatedly to dissolve BTS powder (avoid vigorous shaking), leave it at room temperature for 5 minutes, and then dissolve it again. After BTS (6.7) is completely dissolved, centrifuge it immediately and use 200 µL PCR tubes are aliquoted, 5 µL per tube, stored at -20°C for later use.

6.10
Preparation of CHCA matrix solution. Add solvent I (6.8) to CHCA (6.6) powder to make the final concentration of CHCA 10 mg/mL.

Shake and mix until the solution is clear. Use 1.5 mL centrifuge tubes for aliquots, 50 µL per tube, seal the mouth of the tube with a parafilm, and store at room temperature for later use.

After storage, if a large amount of precipitation occurs, it should be discarded.

7 Main instruments and equipment

7.1
Bench-top centrifuge. the maximum centrifugal force is >= 16 000 g.

7.2
Vortex oscillator.

7.3
Micropipettes. 10 µL, 100 µL,.200 µL, 1000 µL.

7.4
Matrix-assisted laser desorption ionization time-of-flight mass spectrometer.

8.1 Detection flow chart

The MALDI-TOF MS detection process of Bacillus cereus in food is shown in Figure 1.

8.2 Sample preparation, enrichment culture and separation

The preparation, enrichment culture and separation steps of Bacillus cereus test samples in food are carried out in accordance with the method of GB 4789.14.

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