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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 3579-2013

Viability detection of Phytophthora sojae Kaufmann and Gerdemann

大豆疫病菌活性检测方法

(English Translation)

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Foreword

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

Please note that some of the content of this document may involve patents. Distribution of this document Structure does not bear the responsibility to identify these patents.

This standard is proposed and managed by the National Certification and Accreditation Administration Committee.

This standard was drafted. People's Republic of China, Shenzhen CIQ, Shenzhen Inspection and Quarantine Science Research Institute, Guangdong micro Institute of Biology, Chinese Academy of Inspection and Quarantine.

1 Scope

This standard specifies the application of laser scanning confocal microscopy Phytophthora pathogen (PhytophthorasojaeKaufmann Gerdemann) method for detecting the activity.

Assay This standard applies to soybeans and soil carried Phytophthora pathogen.

Principle 2

Application of fluorescent dyes pathogen Phytophthora sojae oospores dyeing process, according to Phytophthora pathogen non-staining activity oospore After the egg plastid broken edge set, oval mass substantially uniform after the dense fluorescence and green are active oospore stained green dyeing effect difference, Using laser scanning confocal microscope to detect spores, analyze its activity situation.

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3.1 Instrument appliances

Laser scanning confocal microscope, microscopes, autoclaves, biological safety cabinet, incubator, refrigerator.

3.2 Reagents

Set acridine orange, phosphate buffered saline (PBS, 0.1mol/μL, pH7.0), water agar medium.

4.1 Get pathogen spores

Under a microscope, the discovery and identification of the pathogen Phytophthora sojae oospores pick sterilized water to prepare a concentration of greater than 1 spore/μL of Spore suspension, microscopy, spare.

4.2 Spore staining

Use the concentration of 100μg/mL (solvent PBS) acridine orange stain fixed with a spore suspension of a volume mixing ratio of 1.20 uniform Together, dark staining 15min at room temperature, 16000g supernatant was centrifuged 1min termination dyeing, sterilizing deionized water once, resuspended spore Spore concentration is greater than 1/μL. Dark for backup.

4.3 Laser scanning confocal microscope to detect activity

4.3.1
Sample preparation will be completed stained slides were mounted upside down on a laser scanning confocal microscope stage, low magnification to find the spores, Go to 63 times the oil microscope, to fine-tune the image within the field of vision clear.

4.3.2
Select the appropriate laser tube (argon ion laser) fluorescence excitation signal, setting an excitation wavelength of fluorescence channels (488nm), collect fluorescent the emission wavelength of the optical signal (505nm), and set a bright field channel as a control.

4.3.3
Choose low-resolution scanning plane rough scan (xy. 512 x 512), based on the scan imaging fluorescence signal strength

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