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National food safety standard - Food microbiological examination - Burkholderia gladioli

食品安全国家标准 食品微生物学检验

唐菖蒲伯克霍尔德氏菌（椰毒假单胞菌酵米面亚种）检验

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1 Scope

This Standard specifies the examination method of Burkholderia gladioli (Pseudomonas cocovenenans subsp. farinofermentans) in the food.

This Standard applies to the examination of Burkholderia gladioli (Pseudomonas cocovenenans subsp. farinofermentans) in the food.

2 Apparatus and materials

2.1 Routine laboratory sterilization equipment.

2.2 Refrigerator: 2 °C~8 °C, -20 °C~-30 °C.

2.3 Constant-temperature incubator: 26 °C±1 °C, 36 °C±1 °C.

2.4 Constant-temperature water bath: 46 °C±1 °C.

2.5 Microscope: 10 times~100 times.

2.6 Homogenizer.

2.7 Centrifuge: 3000 r/min.

2.8 Electronic balance: Sensitivity is 0.1 g.

2.9 Nephelometer.

2.10 Conical flask: Capacity is 100 mL and 500 mL.

2.11 Sterile petri dish: Diameter is 90 mm and 150 mm.

2.12 Sterile transparent cellophane, filter paper.

2.13 Sterile gastric lavage device: 1 mL.

2.14 Microbial biochemical identification system.

2.15 Mice: 18 g~20 g. Each batch of test shall use the same strain of KM or ICR

saline is used as a control. For those agglutinating with multivalent serum, use O-III, O-IV, O-V, O-VI, O-VII, O-VIII factor serum in order, to do test tube agglutination test. According to the test results, determine the somatic antigen type. Those self-coagulating in normal saline cannot be typed. For those which meet the biochemical characteristics but cannot agglutinate with the above serum, the strains need to be retained for further identification.

5.7 Toxicity test

5.7.1 Toxin-producing cultivation

Inoculate the strain, preliminarily identified as Burkholderia gladioli (Pseudomonas cocovenenans subsp. farinofermentans), on a PDA plate; CULTURE at 36 °C±1 °C for 24 h±2 h. USE a sterile inoculation loop to scrape an appropriate amount of lawn; ADD it to a test tube of 3 mL of sterile normal saline; prepare a 1 McFarland (MCF) bacterial suspension (approximately 10^8 CFU/mL). USE a sterile straw to suck up 0.5 mL; DROP it on a potato dextrose semi-solid plate with a diameter of 150 mm spread with sterile cellophane; USE a sterile L bar to spread evenly; incubate at 26 °C±1 °C for 5 d. REMOVE the cellophane that carries bacteria; PLACE the semi-solid plate at 100 °C flowing steam for 30 min of sterilization. After cooling at room temperature, place it in a refrigerator at -20 °C~-30 °C overnight. THAW the frozen semi-solid plate at room temperature; USE a sterile straw to suck out the freeze-thaw solution; filter it through a filter paper into a sterile test tube or conical flask (this is the crude toxin extract); STORE it at 4 °C in the dark.

At the same time, use a potato dextrose semi-solid plate with a diameter of 150 mm spread with sterile cellophane without inoculation of lawn as a negative control. According to the same test method, prepare the negative control crude extract and store at 4 °C in the dark.

5.7.2 Determination of virulence

TAKE the crude toxin extract, or the 5 ~ 10 times concentrated solution after evaporation in a 100 °C water bath; gavage 3 mice, each with 0.5 mL; observe for 7 d. If the strain produces bongkrekic acid, the mice will become ill within 20 min~24 h after gavage. The main symptoms are erected hair, flagging, and restlessness, followed by staggering, numbness, paralysis, convulsions, opisthotonos, rapid breathing, and death.

TAKE the negative control crude extract, or the 5 ~ 10 times concentrated solution after evaporation in a 100 °C water bath; gavage 3 mice, each with 0.5 mL; observe for 7 d. The mice shall survive healthy.

5.7.3 Determination of bongkrekic acid

Appendix A

Media and reagents

A.1 GVC enrichment solution

A.1.1 Potato dextrose water (PD water)

A.1.1.1 Composition

Potato (peeled): 300 g

Dextrose: 20 g

Distilled water: 1000 mL

pH: 7.0±0.2

A.1.1.2 Preparation method

WEIGH 300 g of peeled potatoes and chop them into pieces; ADD 1000 mL of distilled water; BOIL for 10 min~20 min. USE gauze to filter and add distilled water to 1000 mL. ADD dextrose, heat to melt, dispense; autoclave at 121 °C for 20 min.

A.1.2 Gentian violet aqueous solution

A.1.2.1 Composition

Gentian violet: 0.1 g

Distilled water: 100 mL

A.1.2.2 Preparation method

TAKE 0.1 g of gentian violet; USE a small amount of distilled water to dissolve; ADD distilled water, to dilute to 100 mL; STORE it in a brown bottle. Filter and sterilize before use.

A.1.3 Chloramphenicol solution

A.1.3.1 Composition

Chloramphenicol: 20.0 mg

Distilled water: 10 mL

Polymyxin sulfate B: 50000 U

Lincomycin: 30000 U

A.3.4 Preparation method

After heating, melting and cooling the PCFA basal medium to 50 °C, add optional additives; MIX well and pour it into a sterile petri dish for later use.

A.4 Potato dextrose semi-solid agar

A.4.1 Composition

Potato (peeled): 300 g

Dextrose: 20 g

Agar: 5 g

Distilled water: 1000 mL

pH: 7.0±0.2

A.4.2 Preparation method

WEIGH 300 g of peeled potatoes and chop them into pieces; ADD 1000 mL of distilled water; BOIL for 10 min~20 min. USE gauze to filter and add distilled water to 1000 mL. ADD dextrose and agar, heat to melt, dispense; autoclave at 121 °C for 20 min.

A.5 Yolk agar

A.5.1 Composition of the basal medium

Meat infusion: 1000 mL

Peptone: 15 g

Sodium chloride: 5 g

Agar: 25 g~30 g

pH: 7.0±0.2

A.5.2 50% yolk saline suspension

A.5.3 Preparation of yolk agar

Prepare basal medium and pack 100 mL per bottle. Autoclave at 121 °C for 15

A.6.4 Staining method

A.6.4.1 FIX the smear on the flame; ADD dropwise crystal violet staining solution, to stain for 1 min; and use water to wash.

A.6.4.2 ADD dropwise Gram iodine solution for 1 min and use water to wash.

A.6.4.3 ADD 95% ethanol dropwise to decolorize, about 15 s to 30 s, until the staining solution is washed away. Do not over decolorize. USE water to wash.

A.6.4.4 ADD the counterstaining solution dropwise; counterstain for 1 min. USE water to wash, wait to dry; inspect under a microscope.

A.7 Oxidase reagent

A.7.1 Composition

N, N, N', N'-tetramethyl-p-phenylenediamine hydrochloride: 1.0 g

Distilled water: 100.0 mL

A.7.2 Preparation method

Prepare a small amount freshly; STORE in the refrigerator away from light; and use within 7 d.

A.7.3 Test method

TAKE a single characteristic colony; SPREAD it on filter paper moistened with oxidase reagent. If the filter paper does not turn purplish red, purple or dark blue within 10 s, the oxidase test is negative. Otherwise, the oxidase test is positive.

Note: Do not use nickel/chromium materials in the test.

A.8 Hugh-Leifson medium (for O/F test)

A.8.1 Composition

Peptone: 2 g

Sodium chloride: 5 g

Dipotassium hydrogen phosphate: 0.3 g

Agar: 4 g

Dextrose: 10 g

A.9.3.1 Kovac reagent: DISSOLVE 5 g of p-dimethylamino formaldehyde in 75 mL of amyl alcohol; and then slowly add 25 mL of concentrated hydrochloric acid.

A.9.3.2 Ou-bo reagent: DISSOLVE 1 g of p-dimethylaminobenzaldehyde in 95 mL of 95% ethanol. Then slowly add 20 mL of concentrated hydrochloric acid.

A.9.4 Test method

PICK a small amount of culture to inoculate; incubate at 36 °C±1 °C for 1 ~ 2 days; and 4 ~ 5 days if necessary. ADD about 0.5 mL of Kovac reagent; SHAKE the test tube gently. The positive ones will be dark red on the reagent layer. Or add about 0.5 mL of Ou-bo reagent and flow down the tube wall, to cover the surface of the culture solution. The positive ones will be rose red at the liquid surface contact.

Note: Peptone shall be rich in chromochloric acid. After each batch of peptone is purchased, it shall be used after identification with known strains.

A.10 Buffer dextrose peptone water (for MR and V-P tests)

A.10.1 Composition

Dipotassium hydrogen phosphate: 5 g

Polyvalent peptone: 7 g

Dextrose: 5 g

Distilled water: 1000 mL

pH: 7.0±0.2

A.10.2 Preparation method

After melting, correct the pH and pack into test tubes, 1 mL per tube; autoclave at 121 °C for 15 min.

A.11 Methyl red (MR) test

A.11.1 Methyl red reagent

A.11.1.1 Composition

Methyl red: 10 mg

95% ethanol: 30 mL

Agar: 20 g

Distilled water: 1000 mL

0.2% bromothymol blue solution: 40 mL

pH: 7.0±0.2

A.13.2 Preparation method

First dissolve the salt in water; adjust the pH; ADD agar, and heat to melt. Then, add the indicator; MIX well and dispense into test tubes; autoclave at 121 °C for 15 min. MAKE a slant.

A.13.3 Test method

PICK a small amount of agar culture to inoculate; incubate at 36 °C±1 °C for 4 d; observe the results every day. Those which are positive will have colonies growing on the slant. The medium will turn from green to blue.

A.14 Phenylalanine medium

A.14.1 Composition

Yeast extract: 3 g

DI-phenylalanine (or L-phenylalanine 1 g): 2 g

Disodium hydrogen phosphate: 1 g

Sodium chloride: 5 g

Agar: 12 g

Distilled water: 1000 mL

pH: 7.0±0.2

A.14.2 Preparation method

After heating and dissolving, dispense into the test tubes; autoclave at 121 °C for 15 min. MAKE a slant.

A.14.3 Test method

PICK a large number of cultures from the agar slant; TRANSPLANT them on phenylalanine agar; incubate at 36 °C±1 °C for 4 h or 18 h~24 h. ADD dropwise 2 ~ 3 drops of 10% ferric chloride solution and run down from the slant culture.

Agar: 0.35 g~0.4 g

Distilled water: 100 mL

pH: 7.4±0.2

A.16.2 Preparation method

Prepare according to the above components; BOIL to dissolve; CORRECT the pH. Dispense into small test tubes. Autoclave at 121 °C for 15 min. Stand upright and solidify for later use.

Note: It is used for mobility observation and bacteria preservation test.

A.17 Ferrous sulfate agar (for hydrogen sulfide test)

A.17.1 Composition

Beef extract: 3 g

Yeast extract: 3 g

Peptone: 10 g

Ferrous sulfate: 0.2 g

Sodium thiosulfate: 0.3 g

Sodium chloride: 5 g

Agar: 12 g

Distilled water: 1000 mL

pH: 7.4±0.2

A.17.2 Preparation method

HEAT to dissolve and correct the pH; dispense into test tubes. Autoclave at 115 °C for 15 min. Stand upright and solidify for later use.

A.17.3 Test method

PICK the agar culture; puncture along the tube wall; incubate at 36 °C±1 °C for 1 ~ 2 days; observe the results. The culture medium for hydrogen sulfide producers turns black.

A.18 Nutrient gelatin

A.19.2 20% urea solution

A.19.2.1 Composition

Urea: 2 g

Distilled water: 8 mL

A.19.2.2 Preparation method

DISSOLVE 2 g of urea in 8 mL of distilled water, filter and sterilize.

A.19.3 Urea agar

COOL 99 mL of the autoclaved basal medium to 50 °C~55 °C; ADD 1 mL of filtered and sterilized urea solution (final concentration of 2%). The final pH shall be 7.2±0.2. Dispense into sterile test tubes and make a slant for later use.

A.19.4 Test method

PICK the agar culture to inoculate; incubate at 36 °C±1 °C for 24 h; observe the results. Those with urease positive make the culture medium red due to alkali production.

A.20 Arginine test

A.20.1 Composition

Peptone: 5 g

Yeast extract: 3 g

Dextrose: 1 g

Distilled water: 1000 mL

1.6% bromocresol purple-ethanol solution: 1 mL

L-arginine: 5 g

pH: 6.8±0.2

A.20.2 Preparation method

After heating and dissolving the components except L-arginine, dispense 100 mL into each bottle; ADD 0.5 g (0.5%) of L-arginine; adjust the pH to 6.8±0.2. No L-arginine is added to the control medium. Dispense 0.5 mL to each tube; DRIP a layer of liquid paraffin on top; autoclave at 115 °C for 10 min.

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