GB

NATIONAL STANDARD OF

THE PEOPLE'S REPUBLIC OF CHINA

GB 4789.34-2016

National Food Safety Standard - Food Microbiological Examination - Examination of Bifidobacterium

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China Food and Drug Administration.

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Foreword

This Standard replaces GB 4789.34-2012 *National Food Safety Standard - Food Microbiological Examination - Identification of Bifidobacterium*.

Compared with GB 4789.34-2012, the main changes in this Standard are as follows:

- added the counting method for Bifidobacterium;
- added MRS medium;
- modified the application scope of this Standard;
- modified Annex B as optional.

National Food Safety Standard - Food Microbiological Examination - Examination of Bifidobacterium

1 Scope

This Standard specifies the identification and counting method for Bifidobacterium.

This Standard applies to the identification and counting of pure bacteria strain of Bifidobacterium. This Standard applies to the identification of bacteria when the food only contains single Bifidobacterium. This Standard applies to the counting when the food only contains Bifidobacterium, i.e., the food may contain one or more different Bifidobacterium species.

2 Equipment and materials

In addition to microbial laboratory routine sterilization and training equipment, other equipment and materials are as follows:

2.1 Constant temperature incubator: 36°C ± 1°C.

2.2 Refrigerator: 2°C ~ 5°C.

2.3 Balance: resolution of 0.01 g.

2.4 Sterile test tube: 18mm × 180mm, 15mm × 100mm.

2.5 Sterile pipettes: 1mL (with 0.01mL scale), 10mL (with 0.1mL scale) or micro-pipettes (200μL ~ 1000μL) and matching tips.

2.6 Sterile petri dish: 90 mm in diameter.

3 Medium and reagent

3.1 Bifidobacterium culture medium: see A.1.

3.2 PYG medium: see A.2.

3.3 MRS medium: see A.3.

3.4 Methanol: analytically pure.

5 Operation steps

5.1 Sterile requirements

All operating procedures shall follow the sterile procedures.

5.2 Identification of Bifidobacterium

5.2.1 Pure bacteria species

5.2.1.1 Sample processing: semi-solid or liquid species are directly inoculated on Bifidobacterium agar plates or MRS agar plates. For solid bacteria or vacuum freeze-dried bacteria, it shall add an appropriate amount of sterilized saline or other suitable diluent to dissolve the bacteria powder.

5.2.1.2 Vaccination: inoculate on Bifidobacterium agar plates or MRS agar plates. Perform anaerobic culture at 36°C ± 1°C for 48h ± 2h that can be extended to 72h ± 2h.

5.2.2 Food sample

5.2.2.1 Sample processing: take 25.0g (mL) of sample into a sterile conical flask or homogeneous bag containing 225.0mL of physiological saline, homogenizing at 8000r/min ~ 10000r/min for 1min ~ 2min or using slapping homogenizer to beat 1min ~ 2min to make 1:10 sample homogenizing solution. The frozen sample may be thawed at 2°C ~ 5°C for not more than 18h or at a temperature not greater than 45°C for not more than 15min.

5.2.2.2 Inoculation or coating: inoculate the above sample homogenizing solution on a Bifidobacterium agar plate or MRS agar plate OR take 0.1mL of sample homogenizing solution of appropriate dilution to evenly coat on a Bifidobacterium agar plate or MRS agar plate. Perform anaerobic culture at 36°C ± 1°C for 48h ± 2h that can be extended to 72h ± 2h.

5.2.2.3 Pure culture: pick up three or more individual colonies to inoculate on Bifidobacterium agar plates or MRS agar plates. Perform anaerobic culture at 36°C ± 1°C for 48h ± 2h that can be extended to 72h ± 2h.

5.2.3 Identification of bacteria

5.2.3.1 Smear microscopy: pick up Bifidobacterium single colony growing in Bifidobacterium plate or MRS plate for dyeing. Bifidobacterium is Gram-positive, short rod-shaped, slender rod-shaped or spherical, can form a variety of branches or bifurcation and other forms, non-acid, no spores, no power.

5.2.3.2 Biochemical identification: pick up Bifidobacterium single colony

5.3.1.2 Preparation of liquid sample: weigh aseptically 1.0mL of sample; place in 9.0mL of diluent; well mix to make 1:10 sample homogenizing solution.

5.3.2 Food sample

5.3.2.1 Sample processing: take 25.0g (mL) of sample; place in a sterile conical flask or homogeneous bag containing 225.0mL of physiological saline, homogenizing at 8000r/min ~ 10000r/min for 1min ~ 2min, or using slapping homogenizer to beat 1min ~ 2min to make 1:10 sample homogenizing solution. The frozen sample may be thawed at 2°C ~ 5°C for not more than 18h or at 45°C for not more than 15min.

5.3.3 Series dilution and culture

Use a 1mL sterile pipet or micro pipette to prepare 10 times serial sample homogenizing solution, homogenizing at 8000r/min ~ 10000r/min for 1min ~ 2min, or using slapping homogenizer to beat 1min ~ 2min. Dilute once for every increment, i.e., switch a 1mL sterile pipet or pipet head once. According to the estimation of the sample concentration, select two to three sample homogenizing solution of appropriate dilution. When performing 10 times increment dilution, pipette 1.0mL of sample in the sterile plate. Do two plates for each dilution. Meanwhile, respectively pipette 1.0mL of blank diluent into two sterile plates for blank control. Timely pour 15mL ~ 20mL of Bifidobacterium agar medium cooled to 46°C or MRS agar medium (can be placed in 46°C ± 1°C constant temperature water bath for insulation) into the plate, rotate the plate to make it evenly mixed. From sample dilution to plate pouring, it requires 15 min. After agar solidification, flip the plate, perform anaerobic culture at 36°C ± 1°C for 48h ± 2h that can be extended to 72h ± 2h. Count the number of colonies on the plate after incubation.

5.3.4 Colony counting

5.3.4.1 It can be observed with the naked eye, if necessary, with a magnifying glass or colony counter. Record the dilution factor and the corresponding number of colonies. The colony counting is expressed in colony-forming units (CFU).

5.3.4.2 Select the total number of colony colonies counted between 30CFU and 300CFU, and the total number of colonies without flake colonies. Record the number of specific colonies on plates below 30CFU. When it exceeds 300CFU, it can be recorded as countless. The number of colonies per dilution shall be the average of two plates.

5.3.4.3 If one of the plates is growing with large flake colonies, it shall not be used. It shall use the plate without flake colonies as the number of colonies of this dilution. If the number of flake colonies is less than half of the plate, and

Annex A

Medium and reagent

A.1 Bifidobacterium agar medium

A.1.1 Ingredients

Peptone	15.0 g
Yeast extract	20.0 g
Glucose	20.0 g
Soluble starch	0.5 g
Sodium chloride	5.0 g
Tomato extract	400.0 mL
Tween 80	1.0 mL
Liver powder	0.3 g
Agar powder	20.0 g
Adding distilled water to	1000.0 mL

A.1.2 Preparation

A.1.2.1 Preparation of cysteine solution: weigh 0.5 g of cysteine solution, add into 1.0 mL of hydrochloric acid to make cysteine all dissolved and prepare cysteine salt solution.

A.1.2.2 Preparation of tomato extract: wash the fresh tomatoes and scrape them; add the same amount of distilled water and heat in a 100°C water bath, stir for 90 min, then filter with gauze, calibrate to pH 7.0 ± 0.1; after sub-packaging the leaching solution, autoclave at 121°C for 15 min ~ 20min.

A.1.2.3 Preparation: add all components of A.1.1 into distilled water, dissolve by heating, and then add into the cysteine solution, calibrate to pH 6.8 ± 0.1; after sub-packaging the leaching solution, autoclave at 121°C for 15 min ~ 20min.

A.2 PYG liquid medium

A.2.1 Composition

Peptone	10.0 g
Glucose	2.5 g
Yeast	5.0 g
Cysteine-HCl	0.25 g
Salt solution	20.0 mL
Vitamin K1 solution	0.5 mL
Hemoglobin solution 5 mg/mL	2.5 mL
Adding distilled water to	500.0 mL

Annex B

Detection of organic acid metabolites of Bifidobacterium

B.1 Preparation of Bifidobacterium culture medium

Inoculate the Bifidobacterium cultured on Bifidobacterium agar plate or MRS agar plate into PYG liquid medium. Then use non-inoculated PYG liquid medium for blank control, anaerobic, culture at 36°C ± 1°C for 48h.

B.2 Preparation of standard solution

B.2.1 Acetic acid standard solution: accurately pipette 5.7 mL of pure acetic acid, add water and dilute to 100.0 mL, shake and calibrate, prepare about 1.0 mol/L acetic acid standard solution. Calibration method: accurately weigh 3.0 g of acetic acid, add 15.0 mL of water, 2 drops of phenolphthalein indicator solution, use 1.0 mol/mL sodium hydroxide solution for titration, and the titration results shall be corrected by blank test. 1.0 mL of 1 mol/mL sodium hydroxide solution is equivalent to 60.05 mg of acetic acid.

B.2.2 Acetic acid use solution: use water to dilute the calibrated acetic acid standard solution to 20.0 mmol/L.

B.2.3 Lactic acid standard solution: pipette 8.4 mL of analytically pure lactic acid, add water to dilute to 100.0 mL, shake, calibrate, and prepare 1.0 mol/L lactic acid standard solution. Calibration method: accurately pipette 1.0 g of lactic acid, add 50.0 mL of water, add 25.0 mL of 1 mol/mL sodium hydroxide titration solution, boil 5min, add 2 drops of phenolphthalein indicator solution, and use 0.5 mol/mL sulfuric acid titration for titration, and titration results shall be corrected by blank test. 1.0 mL of 1 mol/mL sodium hydroxide solution is equivalent to 90.08 mg of lactic acid.

B.2.4 Lactic acid use solution: use water to dilute lactic acid standard solution to 20.0 mmol/L.

B.3 Method

B.3.1 Treatment of acetic acid

Take 2.0ml ~ 3.0mL of Bifidobacterium culture medium into 10mL centrifuge tube, add 0.2mL of 50% sulfuric acid solution (volume fraction), mix, add 2.0mL of acetone, mix and add excess sodium chloride, shake for 1min , then add 2.0mL of ether, shake for 1min, centrifuge at 3000 r/min for 5min. Transfer the supernatant to another tube. Use 2.0 mL of acetone and 2.0 mL of diethyl ether to extract the lower solution twice. Combined organic phases

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