NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.35-2023

National food safety standard - Food microbiological examination - Lactic acid bacteria

Issued on: September 6, 2023 **Implemented on: September 6, 2024**

Issued by: NHC; SAMR

Table of Contents

Foreword

This Standard replaces GB 4789.35-2016 "National food safety standard -- Microbiological examination of food -- Examination of lactic acid bacteria".

Compared with GB 4789.35-2016, the main changes in this Standard are as follows.

- Add real-time fluorescence PCR method as an optional method;

- Modify the definition of lactic acid bacteria, sample preparation, and culture time;

- Modify the description of the enumeration method for streptococcus thermophilus and lactobacillus;

- Modify some culture medium components, stock solution concentrations and preparation methods.

National Food Safety Standards -- Food Microbiology Testing -- Lactobacillus Testing

1 Scope

This Standard specifies the testing methods for lactic acid bacteria in foods containing lactic acid bacteria.

This Standard is applicable to the inspection of lactic acid bacteria in foods containing

active lactic acid bacteria.

2.1 lactic acid bacteria

A general name for a group of bacteria that ferment sugars and mainly produce large amounts of lactic acid. It is a bacterium that cannot liquefy gelatin, does not produce indole, is Gram-positive, non-motile, non-spore-bearing, catalase-negative, nitrate reductase-negative and cytochrome oxidase-negative.

The lactic acid bacteria in this Standard are mainly Lactobacillus, Bifidobacterium and Streptococcus thermophilus.

3 Equipment and materials

In addition to the routine sterilization and culture equipment in the microbiology laboratory, other equipment and materials are as follows.

3.2 Anaerobic culture device. anaerobic incubator, anaerobic tank, anaerobic bag or

device that can provide equivalent anaerobic effect.

3.4 Homogenizer and sterile homogenization bag, homogenization cup or sterilized

mortar.

4.3 Li-Mupirocin and Cysteine Hydrochloride modified MRS agar medium. see A.3 in

Annex A.

4.4 MC (Modified Chalmers) agar medium. see A.4 in Annex A.

4.5 0.5% sucrose fermentation tube. see A.5 in Annex A.

4.6 0.5% cellobiose fermentation tube. see A.5 in Annex A.

4.7 0.5% maltose fermentation tube. see A.5 in Annex A.

4.8 0.5% mannitol fermentation tube. see A.5 in Annex A.

4.9 0.5% salicin fermentation tube. see A.5 in Annex A.

4.10 0.5% sorbitol fermentation tube. see A.5 in Annex A.

4.11 0.5% lactose fermentation tube. see A.5 in Annex A.

5 Inspection procedures

The inspection procedures for lactic acid bacteria are shown in Figure 1.

6.1.2 The diluent should be fully preheated at 36 degrees C+/-1 degrees C for 15 min~30 min before

testing.

6.1.3 Frozen samples can be thawed at 2 degrees C~5 degrees C first. The time does not exceed 18 h.

It can also be defrosted at a temperature not exceeding 45°C. The time does not exceed

6.1.4 Solid and semi-solid samples. Weigh 25 g of sample aseptically. Place in a sterile

homogenizing cup containing 225 mL of diluent. Homogenize at 8000 x g ~ 10000 x g for 1 min ~ 2 min to prepare a 1.10 sample homogeneous solution. Or place in a sterile homogenization bag with 225 mL of diluent. Use a slap homogenizer to beat for 1 min ~ 2 min to prepare a 1.10 sample homogeneous solution.

6.1.5 Liquid sample. The liquid sample should be shaken thoroughly first. Then use a

sterile straw to take 25 mL of the sample and put it into a sterile Erlenmeyer flask containing 225 mL of diluent (an appropriate number of sterile glass beads are preset in the bottle) or a homogeneous bag. Shake thoroughly or beat with a slap homogenizer for 1 min ~ 2 min to prepare a 1.10 sample homogeneous solution.

6.1.6 Food samples containing lactic acid bacteria that have been processed by special

technologies (such as embedding technology) should be effectively pre-treated under the corresponding technical/process requirements.

6.3.1 Total number of lactic acid bacteria

The selection of culture conditions for counting the total number of lactic acid bacteria

and the explanation of the results are shown in Table 1.

6.3.2 Bifidobacteria counting

Based on the estimate of the bifidobacteria content of the sample to be tested, 2 to 3 consecutive appropriate dilutions are selected. Pipette 1 mL of sample homogeneous solution for each dilution into a sterilized plate. Make two plates for each dilution. After the dilution is transferred to the plate, pour 15 mL ~ 20 mL of mupirocin lithium salt and cysteine hydrochloride modified MRS agar medium that have been cooled to 48 degrees C~50 degrees C into the plate. Swirl the dish to mix evenly.

6.3.3 Streptococcus thermophilus counting

Based on the estimate of the viable number of streptococcus thermophilus in the sample to be tested, 2 to 3 consecutive appropriate dilutions are selected. Pipette 1 mL of sample homogeneous solution for each dilution into a sterilized plate. Make two plates for each dilution. After the diluent is transferred to the plate, pour 15 mL ~ 20 mL of MC agar medium cooled to 48 degrees C~50 degrees C into the plate in time. Swirl the dish to mix

evenly. After the culture medium solidifies, place it upside down for aerobic culture at 36°C+/-1°C. According to the growth characteristics of streptococcus thermophilus, culture is generally selected for 48 h. If the colony does not grow or grows small, you can choose to culture it for 72 h. The colony characteristics of streptococcus thermophilus on the MC agar medium plate are. the colonies are medium to small, red colonies with neat and smooth edges, 2 mm+/-1 mm in diameter, and the back of the colonies is pink.

6.4 Colony counting

See the colony counting part of GB 4789.2.

6.5 Result expression

See the calculation method part of GB 4789.2.

6.6 Report of colony counting