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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 13093-2023

Replacing GB/T 13093-2006

Determination of bacterial count in feeds

饲料中细菌总数的测定

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1 Scope

GB/T 13093-2023 is the Chinese national standard on determination of bacterial count in feeds, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 6 August 2023 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 March 2024. Classification: ICS 65.120, CCS B46. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This document describes the plate count method for the detection of the bacterial count in feed. This document is applicable to the determination of the bacterial count in compound feed, concentrated feed, concentrate supplements, and feed ingredients of animal and plant origin.

2 Normative references

The provisions of the following documents constitute the essential clauses of this document through normative references in this text. Among them, for referenced documents with dates, only the versions corresponding to the dates are applicable to this document; for referenced documents without dates, the latest versions (including all amendments) are applicable to this document.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB/T 20195 Animal feeding stuffs - Preparation of test samples

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 bacterial count The number of colonies obtained per gram or per milliliter of the specimen after the feed specimen is processed and cultured under certain conditions (such as using a specific culture medium, culture temperature, and time). NOTE: The number of colonies is expressed in colony-forming units (CFU).

4 Principle

After the feed sample is properly treated and diluted, the microorganisms in it are fully dispersed into single cells. A certain amount of the diluted sample solution is inoculated on a plate. After cultivation, each single cell grows and multiplies to form colonies visible to the naked eye, that is, a single colony shall represent a single cell in the original sample; the number of colonies is counted, and the number of bacteria in the feed can be calculated based on the dilution multiple and the sampling inoculation amount.

5 Reagents or materials

Unless otherwise specified, only analytical-grade reagents are used.

5.1 Water: The grade 3 specified in GB/T 6682.

5.2 Sodium hydroxide solution (1 mol/L): Weigh 20 g of sodium hydroxide, dissolve it in water, cool to room temperature, and make up to 500 mL with water.

5.3 Hydrochloric acid solution (1 mol/L): Measure 42 mL of concentrated hydrochloric acid, dilute to 500 mL with water, and shake well.

5.4 Phosphate buffer stock solution: Weigh

34.0 g potassium dihydrogen phosphate and dissolve it in 500 mL water. Adjust the pH to

7.2 with about 175 mL of 1 mol/L sodium hydroxide solution, dilute to 1000 mL with water,

mix well, and store at 2 °C~8 °C.

5.5 Sterile phosphate buffer: Take

1.25 mL of phosphate buffer stock solution (5.4), dilute to 1000 mL with water, dispense into suitable containers or 9 mL per tube, and sterilize by autoclaving at 121 °C for 15 min.

5.6 Sterile physiological saline: Weigh

8.5 g of sodium chloride, dissolve it in water, and make up to 1000 mL. Dispense into suitable containers or 9 mL per tube and sterilize by autoclaving at 121 °C for 15 min.

5.7 Plate count agar medium: Prepare according to Appendix A.

5.8 Sterile liquid paraffin: Take liquid paraffin and sterilize it by autoclaving at 121 °C for 20 min.

5.9 Sterile Tween 80: Take Tween 80 and sterilize it by autoclaving at 121 °C for 20 min.

6 Instruments and equipment

6.1 Constant temperature incubator: The temperature control accuracy is ±1 °C.

6.2 Slap homogenizer.

6.3 Constant temperature device: The temperature control accuracy is ±2 °C.

8.1.2.2 Follow the procedure in

8.1.2.1 to make 10-fold incremental dilutions. The sterile pipette or micropipette tip shall be replaced after each incremental dilution.

8.1.2.3 According to the estimation of the contamination status of the sample, select 2 ~3 sample homogenates of appropriate serial dilutions, and pipette 1 mL of sample homogenate of each dilution into a sterile plate; make two plates for each dilution. At the same time, pipette 1 mL of sterile phosphate buffer or sterile physiological saline into a sterile plate as a blank control.

8.2 Cultivation After the dilution is transferred to the plate, promptly pour 15 mL~20 mL of the culture medium cooled to 46 °C~50 °C (can be placed in a 48 °C±2 °C constant temperature device) into the plate, and carefully rotate the plate to fully mix the sample and the culture medium. The time from diluting the sample to pouring the culture medium shall not exceed 30 min. If it is estimated that the sample may diffusely grow on the surface of the culture medium, after the culture medium is completely solidified, a thin layer of plate count agar medium (about 4 mL) cooled to 48 °C±2 °C can be covered on the solidified agar surface. After the agar solidifies, invert the plate horizontally and culture it in a 36 °C±1 °C constant temperature incubator for 48 h±2 h.

8.3 Bacterial colony counting

8.3.1 When counting bacterial colonies, observation can be made with the naked eye. If necessary, a magnifying glass or colony counter can be used to check to prevent omissions. Bacterial counts are expressed in colony-forming units (CFU).

8.3.2 Select plates with colony counts between 30 CFU ~ 300 CFU and no spreading colony growth for counting. For plates with less than 30 CFU, record the specific colony count; for plates with more than 300 CFU, record as TNTC (too numerous to count).

8.3.3 When one of the two plates has large flake-like colonies growing, the plate without large flake-like colonies shall be selected for colony counting; if the flake-like colonies are less than half of the plate, and the colonies on the other half are evenly distributed, the half of the plate with evenly distributed colonies shall be selected for colony counting, and the count result multiplied by 2 represents the colony number on this plate.

8.3.4 If there are chain-like growths without clear boundaries between colonies on the plate, each single chain shall be counted as one colony.

8.3.5 If there is colony growth on the blank control, the test result will be invalid.

9.1 Calculation of bacterial count

9.1.1 If the number of colonies on only one dilution plate is within the appropriate counting range, calculate the average value of the colony counts on the two plates and then multiply the average value by the corresponding dilution multiple to obtain the bacterial count per gram or per milliliter of the sample. See Example 1 in Appendix B.

9.1.2 If the colony counts on two serial dilution plates are within the appropriate counting range, calculate it according to formula (1): where: N -- The bacterial count in the sample; C -- The number of colonies on the plates (the plates on which the number of colonies is in the appropriate range); n1 -- The number of plates of the first dilution (low dilution multiple); n2 -- The number of plates of the second dilution (high dilution multiple); d -- Dilution factor (first dilution). The calculated result is the bacterial count per gram or per milliliter of the sample, see Example 2 in Appendix B.

9.1.3 If the colony counts on all dilution plates are greater than 300 CFU, count the plate with the highest dilution and record the other plates as TNTC. The result is calculated by multiplying the average colony count by the highest dilution multiple, see Example 3 in Appendix B.

9.1.4 If the colony counts on the plates of all dilutions are less than 30 CFU, the calculation shall be based on the average colony count of the lowest dilution multiplied by the dilution multiple, see Example 4 in Appendix B.

9.1.5 If no bacteria grow on any dilution plate, calculate by multiplying the lowest dilution