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Replace GB/T 13092-1991

Enumeration of molds count in feeds

饲料中霉菌总数的测定

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Enumeration of molds count in feeds

1 Scope

This standard specifies the method for the determination of the molds count in feeds.

This standard applies to the determination of the molds count in feeds.

2 Normative references

The following documents contain the provisions which, through normative reference in this document, constitute the essential provisions of this document. For the dated referenced documents, only the versions with the indicated dates are applicable to this document; for the undated referenced documents, only the latest version (including all the amendments) is applicable to this document.

GB/T 4789.2-2003 Microbiological examination of food hygiene - Detection of aerobic bacterial count

GB/T 6682-1992 Water for analytical laboratory use - Specification and test methods

GB/T 14699.1 Feeding stuffs - Sampling

3 Terms and definitions

The following terms and definitions apply to this standard.

3.1 molds count

The total number of molds contained in 1 g of the sample obtained after the feed sample is treated and cultured under certain conditions.

4 Principles

According to the physiological characteristics of the mold, a medium that is suitable for the growth of the mold but not suitable for the growth of the bacteria shall be selected, and the number of the mold shall be determined by using the plate count method.

5 Equipment and materials

5.1 Analytical balance: The sense quantity shall be 0.001 g.

5.2 Constant temperature incubator: (25~28) °C±1 °C.

5.3 Refrigerator: an ordinary refrigerator.

5.4 Autoclave: 2.5 kg.

5.5 Water bath: (45~77) °C±1 °C.

5.6 Oscillator: reciprocating.

5.7 Micro mixer: 2900 r/min.

5.8 Sterilized glass Erlenmeyer flask: 250 mL, 500 mL.

5.9 Sterilized test tube: 15 mm×150 mm.

5.10 Sterilized plate: 90 mm in diameter.

5.11 Sterilized pipette: 1 mL, 10 mL.

5.12 Sterilized glass beads: 5 mm in diameter.

5.13 Sterilized jar: 100 mL, 500 mL.

5.14 Sterilized metal spoons, knives, etc.

6 Media and reagents

Unless otherwise specified, the reagents used are all analytical grade; the water shall conform to the third-grade water specified in GB/T 6682-1992.

6.1 Salt Czapek Dox Agar: See Appendix A for the preparation method.

6.2 Diluent: Weigh 8.5 g of sodium chloride, and dissolve it in 1000 mL of distilled water; after sub-packaged, autoclave at 121 °C for 30 min.

6.3 Disinfectants commonly used in laboratories.

8 Preparation of samples

Sampling shall be carried out in accordance with GB/T 14699.1 method, and special attention must be paid to selecting representative samples and to avoiding contamination during sampling. First, prepare the sterilized containers and sampling tools, such as sterilized kraft paper bags or jars, metal spoons, and knives. On the basis of hygienic investigation, take a representative sample, grind it, and sieve it through a sifter with an aperture of 0.45 mm; then, reduce the sample to 250 g with the quartering method. The sample shall be tested as soon as possible, otherwise, the sample shall be placed in a dry place at a low temperature.

9 Analysis steps

9.1 Weigh 25 g (or 25 mL) of the test sample by sterile operation, put it into a glass Erlenmeyer flask containing 225 mL of sterilized diluent, place it on an oscillator, and shake it for 30 min to obtain a 1:10 diluent.

9.2 Pipette 10 mL of the 1:10 diluent with a sterilized pipette, inject it into a test tube with glass beads, place it on a micro-mixer and mix for 3 minutes; or inject it into a test tube, blow and suck it 50 times repeatedly with a 1 mL sterilized pipette with a rubber nipple to disperse the mold spores.

9.3 Take 1 mL of 1:10 diluent, inject it into a test tube containing 9 mL of the sterilized diluent, and use another pipette to blow and suck it 5 times; then, this solution is a 1:100 diluent.

9.4 Prepare 10-fold incremental diluents according to the above operation sequence, and change a 1 mL sterilized pipette for each dilution. According to the estimation of the degree of contamination of the sample, choose 3 diluents with appropriate dilution, pipette 1 mL from each diluent during preparing 10-fold incremental diluents, add it to the sterilized plate, and make 2 plates for each dilution; then, inject the Salt Czapek Dox Agar that has cooled to about 45 °C into the plates, mix well; after the agar solidifies, place them upside down in a constant temperature incubator at (25~28) °C±1 °C, observe after 3 days of cultivation, and observe for one week.

10 Calculation

10.1 Usually, the plate with molds between 10~100 is selected for counting. The average number of molds in 2 plates with the same dilution is multiplied by the dilution factor, the result is the molds count contained in each gram (or per milliliter) of the sample.

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