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Feed additives - Part 5: Live microorganisms - Saccharomyces cerevisiae

饲料添加剂 第 5 部分:微生物 酿酒酵母

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Feed additives - Part 5: Live microorganisms - Saccharomyces cerevisiae

1 Scope

This document specifies technical requirements, sampling, test methods, inspection rules, labeling, packaging, transportation and storage for Saccharomyces cerevisiae, a feed additive.

This document is applicable to the feed additive Saccharomyces cerevisiae prepared by liquid fermentation, dehydration and drying with Saccharomyces cerevisiae as strain.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB 4789.15-2016, *National food safety standard - Food microbiological examination - Enumeration of moulds and yeasts*

GB/T 6435, *Determination of moisture in feedstuffs*

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

GB/T 8170, *Rules of rounding off for numerical values and expression and judgement of limiting values*

GB 10648, *Feed label*

GB/T 13079, *Determination of total arsenic in feeds*

GB/T 13080, *Determination of lead in feeds - Atomic absorption spectrometry*

GB/T 13081, *Determination of mercury in feeds*

GB/T 13082, *Determination of cadmium in feeds*

GB/T 13091, *Determination of Salmonella in feeds*

possible foreign contamination.

5.2 Sampling method

5.2.1 Samples shall be collected in the same batch. The sampling amount of each sample shall meet the requirements of microbiological indicator inspection. Generally, it is not less than 500g.

5.2.2 For products with independent packaging less than or equal to 500g, take the complete package.

5.2.3 For products larger than 500g individually packaged, a sterile sampler shall be used to take appropriate samples from different parts of the same package. Place them in the same sterile sampling container as one sample.

5.3 Storage and transportation of collected samples

5.3.1 Samples shall be sent to the laboratory for inspection as soon as possible.

5.3.2 Samples shall be kept intact during transportation.

5.3.3 Samples shall be stored close to their original storage temperature. Or take necessary measures to prevent changes in the number of microorganisms in the sample.

6 Test method

Unless otherwise specified, only the reagents confirmed as analytically pure are used in the analysis, and the water meets the requirements of grade 3 water in GB/T 6682.

6.1 Sensory inspection

Take an appropriate amount of specimen. Place on a clean white piece of paper. Observe its shape, color, presence or absence of impurities under natural light. Smell its odor.

6.2 Strain identification

Carry out in accordance with Annex A. Use panel method as the arbitration method.

6.3 Yeast viable cell count

Carry out in accordance with Annex B.

6.4 Moisture

Carry out in accordance with GB/T 6435.

6.5 Total arsenic

Carry out in accordance with GB/T 13079.

6.6 Lead

Carry out in accordance with graphite furnace atomic absorption spectrometry in GB/T 13080.

6.7 Mercury

Carry out in accordance with GB/T 13081.

6.8 Cadmium

Carry out in accordance with GB/T 13082.

6.9 Salmonella

Carry out in accordance with GB/T 13091.

7 Inspection rules

7.1 Batching

A batch of products refer to the products of the same specification produced in the same material and in the same production process through continuous production or in the same shift. But each batch of products shall not exceed 50t.

7.2 Exit-factory inspection

Appearance and properties, yeast live cell count, and moisture are the inspection items of the exit-factory inspection.

7.3 Type inspection

Type inspection items are all items specified in Chapter 4 of this document. Under normal production conditions, type inspection shall be carried out at least once a year. Type inspection shall also be carried out in one of the following situations:

a) When the product is stereotyped and put into production;

b) When there is a major change in the production process, formula or source of main raw materials, which may affect the quality of the product;

c) When production is stopped for more than 3 months and production is resumed;

d) When there is a significant difference between the exit-factory inspection results and the last type inspection results;

Annex A

(normative)

Identification method for Saccharomyces cerevisiae

A.1 Morphological identification

A.1.1 Culture medium

A.1.1.1 Wort liquid medium: The wort is diluted with water to 10°Bx~15°Bx (Bahrain Brix Meter). Sterilize by autoclaving at 115°C for 15min.

A.1.1.2 Wort agar medium: The wort is diluted with water to 10°Bx~15°Bx (Bahrain Brix Meter). Add 2% agar powder. Sterilize by autoclaving at 115°C for 15min.

A.1.1.3 Sporulation medium: 0.1% of glucose, 0.18% of potassium chloride, 0.25% of yeast juice, 0.82% of sodium acetate, 2% of agar. Use distilled water to prepare. Put in a tube. Sterilize by autoclaving at 115°C for 15min. Shelve bevel.

A.1.1.4 Potato dextrose agar: 200g of peeled potato, 20g of glucose, 20g of agar, 1L of tap water. Clean the potatoes. Remove the peel. Cut into small pieces. Immediately put into water to avoid oxidation. Boil 30min. Filter by gauze. Add water to the filtrate to 1L. Add glucose and agar. Subpack into conical flasks or test tubes after they are dissolved. Sterilize by autoclaving at 115°C for 20min.

A.1.2 Growth in wort liquid medium

After 3 days of static culture at 28°C, bacteria settle tightly at the bottom in liquid medium. The culture medium is clear. No biofilm is formed. Take a small number of bacteria. Observe under a 400x microscope. Cells are oval or round, single or double, occasionally in clusters, cell budding. The ratio of cell length to width is 1~2. Cell size is divided into three types: large, medium and small. The size of large cells is (4.5μm~10μm) × (7.0μm~21μm). The size of medium cells is (3.5μm~8μm) × (5.0μm~17.5μm). The size of small cells is (2.5μm~7μm) × (4.5μm~11μm).

A.1.3 Growth on wort agar medium

After culturing at 28℃ for 3 days, the colonies are large and moist, slightly raised or flat, milky white. The surface is smooth and wrinkle-free. The edges are neat.

A.1.4 Growth on potato dextrose agar

After culturing at 28℃ for 3 days, grow on potato agar medium. There is no pseudohyphae or relatively developed but atypical pseudohyphae.

Annex B

(normative)

Determination method for yeast viable cell count

B.1 Method One -- Plate Method (Arbitration Method)

Test according to Method One of GB 4789.15-2016. When testing, the temperature of "adding 225mL of sterile diluent" in 5.1.1 of GB 4789.15-2016 is 37℃~40℃.

B.2 Method Two -- Dyeing Method

B.2.1 Principle

Live yeast can immediately reduce and decolorize the methylene blue staining solution that enters the cell. It is not stained, while dead yeast is stained blue. The number of viable cells can be counted by microscopic observation.

B.2.2 Reagents or materials

B.2.2.1 Sterile physiological saline: 0.85% sodium chloride solution.

B.2.2.2 Methylene blue staining solution: Take 0.025g of methylene blue, 0.042g of potassium chloride, 0.048g of calcium chloride hexahydrate, 0.02g of sodium bicarbonate, 1.0g of glucose. Add sterile saline to dissolve them. Set the volume to 100mL. Seal. Store at room temperature.

B.2.3 Instruments and equipment

B.2.3.1 Microscope: The magnification is above 400.

B.2.3.2 Hemocytometer.

B.2.3.3 Special cover glass for hemocytometer.

B.2.3.4 Analytical balance: The accuracy is 0.1mg.

B.2.3.5 Constant temperature water bath: The temperature control accuracy is ±0.5℃.

B.2.4 Test steps

B.2.4.1 Weigh 0.1g of sample, accurate to 0.0002g. Accurately add 20mL of 37℃~40℃ sterile saline. Oscillate to fully disperse. Activate in a constant temperature water bath at 32°C for 1h.

B.2.4.2 Shake the activation solution evenly. Take 0.1mL of yeast activation solution

into a test tube. Add 0.9mL of methylene blue staining solution. Shake well. Stain at room temperature for 10min.

B.2.4.3 Place the coverslip on the hemocytometer chamber. Cover it tightly on the hemocytometer. Take 0.02mL of the stained bacterial solution (B.2.4.2) to the junction of the hemocytometer and the coverslip. Let the bacterial liquid be automatically sucked into the counting chamber. There shall be no air bubbles in the bacterial solution. After standing for 1 min, use a microscope to observe the counts.

NOTE 1: Counting boards usually come in two sizes. One is that there are 16 medium grids in 1 large grid. 1 medium grid is divided into 25 small grids, i.e., the size of 16×25. With this specification counting board, take the upper left, lower left, upper right and lower right four middle grids (that is, 100 small grids) for counting. The other is that 1 large grid is divided into 25 medium grids. A medium grid is divided into 16 small grids, that is, the size of 25×16. With this kind of counting board, in addition to the upper left, lower left, upper right, and lower right four middle boxes, it is necessary to add a middle grid (that is, 80 small grids) in the center for counting.

NOTE 2: Do not slide the coverslip after it is in place, otherwise the cells will also move. It is horizontal when counting after instillation.

B.2.4.4 After finding the square with 10× objective lens and 16× eyepiece, change to 40× objective lens. Slightly adjust for the clearest field of view. Start counting. When the cells are on the grid line, the counting principle: counting up but not counting down, counting left but not right. When budding is counted, cells that exceed one-half of the mother cell are counted as cells. Those less than half are ignored. Dead cells are stained blue. Live cells are colorless. Only viable cells are counted.

NOTE 1: Saccharomyces cerevisiae cells are oval or elliptical when observed under a microscope. The size is (5μm~7.5μm) × (7.5μm~10μm). Obvious nuclei can be seen in the cells.

NOTE 2: Count each sample twice. Take the arithmetic mean.

B.2.5 Result calculation

The number of viable cells per gram of specimen X_1, is counted in quantity. The value is expressed in cells/g. Calculate according to formula (B.1):

$$X_1 = \frac{A \times 400 \times 10^4 \times 20 \times 10}{m \times N} \quad \cdots\cdots (B.1)$$

Where,

X_1 - The number of viable cells per gram of sample, in cells per gram (cells/g);

A - The number of viable cells in the counted cells, in cells;

m - The amount of weighed sample, in grams (g);

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