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**Microorganism feed additives *Bacillus Licheniformis*
(English Translation)**

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1 Scope

This Standard specifies the requirements, test methods, inspection rules, labeling, packaging, transportation, storage and shelf life requirements for the microorganism feed additive product of *Bacillus Licheniformis*.

This Standard applies to solid and liquid feed additives containing *Bacillus Licheniformis*.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, all subsequent amendments (not including errata content) or revisions do not apply to this standard. However, parties to agreements that are based on this Standard are encouraged to study whether the latest versions of these documents can be used. For undated references, the latest edition applies to this Standard.

GB/T 4789.3, Microbiological examination of food hygiene - Detection of Coliform bacteria

GB/T 4789.5, Microbiological examination of food hygiene - Examination of *Shigella*

GB/T 4789.10, Microbiological examination of food hygiene - Examination of

Staphylococcus aureus

GB/T 5918, Determination of mixing homogeneity for feed products

GB/T 6435, Determination of moisture in feedstuffs

GB 10648, Feed label

GB 13078, Hygienical standard for feeds

GB/T 13079, Determination of total arsenic in feeds

GB/T 13080, Determination of lead in feeds

GB/T 13081, Determination of mercury in feeds

GB/T 13082, Determination of cadmium in feeds

GB/T 13091, Determination of Salmonella in feeds

GB/T 13092, Enumeration of molds count in feeds

GB/T 13093, Determination of bacterial count in feeds

GB/T 14699.1, Animal feeding stuffs - Sampling

GB/T 17480, Determination of aflatoxin B1 in animal feeding stuffs - Enzyme- linked immunosorbent assay

3 Terms and definitions

3.1

Bacillus Licheniformis

A species belonging to the family Bacillaceae and the genus *Bacillus*. The cells are rod-shaped, Gram-positive, spore-forming and central. It can resist adverse environmental conditions and is used as a microbial feed additive in the feed industry.

3.2

Number of contaminating microorganisms

The total number of bacterial and mold colonies other than *Bacillus Licheniformis* grown on the selective medium.

3.3

Contamination rate

The percentage of the number of contaminating microorganisms in the sum of the number of *Bacillus Licheniformis* and the number of contaminating microorganisms.

4.1.1 Cell morphology

4.1.1.1

Staining. Gram staining shall be positive.

4.1.1.2

Morphological characteristics. The bacterial cells (vegetative cells) shall be straight rod-shaped, arranged singly, in pairs or in short chains, with a size of $0.6\ \mu\text{m}\sim 0.8\ \mu\text{m} \times 1.5\ \mu\text{m}\sim 3.5\ \mu\text{m}$, and with blunt and rounded ends. Spores shall be present. The spores shall be nearly oval-shaped and central, with a size of $0.8\ \mu\text{m} \times 1.3\ \mu\text{m}$. The

4.3 Moisture (solid microbial agent)

Not higher than 9.0%.

4.4 Particle size (solid microbial agent)

It shall pass through the test sieve of SSW0.400/0.250 mm.

4.5 Microbial content

The number of *Bacillus Licheniformis* shall be $\geq 1 \times 10^9$ CFU/mL or g.

4.6 Hygienic indicators

The *Bacillus Licheniformis* feed additive shall comply with the requirements in Table 2.

Table 2 - Hygienic indicators of *Bacillus Licheniformis* feed additive

5.1 Sampling

Carry out sampling according to the provisions of GB/T 14699.1. During sampling, special attention shall be paid to the representativeness of the sample and the avoidance of contamination during sampling. Prepare sterilized containers and sampling tools first, such as sterilized kraft paper bags or wide-mouth bottles, metal spoons and knives.

Based on hygienic investigation, take representative samples. Test the sample immediately after collection.

5.2 Sensory test

Place a certain amount of sample (250 g for solid sample and 250 mL for liquid sample) into a colorless glass cup, and test by visual observation and nose smelling.

5.3 Moisture determination

Carry out determination according to GB/T 6435.

5.4 Particle size (solid additive) detection

Perform according to the provisions of GB/T 5918.

5.5 Detection of *Bacillus Licheniformis*

5.5.1

Examination of cell morphology.

5.5.1.1

Staining. Perform Gram staining and spore staining.

5.5.1.2 Morphological examination. Use nutrient agar. After culturing at 30 °C for 48

h, take bacterial cells in a sterile environment, smear, stain, and the observe cell morphology by an optical microscope.

5.5.2 Examination of colony characteristics. On nutrient agar, Sauton's agar and beef

extract agar plates, after culturing at 30 °C for 48 h, observe the color, morphology and pigment of colonies.

5.5.3 Examination of physiological and biochemical characteristics. Refer to the relevant contents of Bergey's Manual of Systematic Bacteriology, Vol. II and Manual for Systematic Identification of Common Bacteria.

5.5.4 Viable cell count

5.5.4.1

The dilution pour plate method shall be adopted. Prepare different concentration dilutions according to the ten-fold dilution method. Separately take a certain volume of each dilution and place into sterile culture dishes. Each dilution shall be repeated three times. Pour the nutrient agar medium, which has been melted in advance and cooled to approximately 50 °C, into each culture dish at about 12 mL. After mixing evenly and solidification, culture the plates upside down for 24 h, and carry out colony counting.