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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 19169-2026

Replacing GB 19169-2003

Spawn of Auricularia heimuer

黑木耳菌种

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1. Structure and Drafting Rules of Standardization Documents". Drafting. This document replaces GB 19169-2003 "Black Fungus Strains". Compared with GB 19169-2003, the main differences are structural adjustments and editorial changes. In addition, the main technological changes are as follows:

- a) The "Container Requirements" have been changed (see Chapter 4, 4.1.1, 4.2.1, and 4.3.1 of the 2003 edition);
- b) Added "species requirements" (see 5.1.1);
- c) The "Biological Requirements" have been amended (see 5.1.2, 4.1.3 of the 2003 edition);
- d) "Culture medium" has been added (see 5.2.1);
- e) The "sensory requirements" have been amended (see 5.2.2, 5.3.1, 5.4.1; 4.1.2, 4.2.2, 4.3.2 in the 2003 edition);

- f) Increased "strain viability" (see 5.2.4, 5.3.3, 5.4.4);
- g) Quality requirements for "liquid cultures" have been added (see 5.4);
- h) The "Test Methods" have been changed (see Chapter 6, Chapter 6 of the.2003 edition);
- i) The "Inspection Rules" have been amended (see Chapter 7, Chapter 7 of the.2003 edition);

5.1 Basic Requirements

5.1.1 Species Requirements Mother culture, primary culture, cultivar, and liquid spawn should all meet the requirements of the varietal characteristics. Variety characteristics include sensitivity to temperature, humidity, pH, and light. And oxygen requirements, as well as agronomical factors such as stress resistance, high yield, ear emergence time, ear emergence tide number, cultivation cycle, commercial quality, and cultivation habits. The condition should meet the requirements of NY/T 2588.

5.1.2 Biological Requirements It should comply with the requirements in Table 2.

5.2 Requirements for the mother breed

5.2.1 Culture medium The culture medium should be poured into the test tube to fill 1/5 to 1/4 of the total volume. After making the slant, the top of the culture medium should be 40 mm from the bottom edge of the sealing material. 50mm; the thickness of the culture medium in the petri dish is 3mm~6mm.

5.2.2 Sensory Requirements

5.2.3 Mycelial growth rate On potato dextrose agar (PDA) medium (prepared according to Appendix A, A.1), at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ Under culturing conditions, the mycelial growth rate was

5.2.4 Strain Viability Mycelial blocks were inoculated on PDA medium, and the germination time of the strain was no more than 72 hours at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

5.3 Original and Cultivated Varieties

5.3.1 Sensory Requirements It should meet the requirements of Table 4, and the culture medium for the original strain should be in close contact with the inner wall of the container, while the culture medium for the cultivated strain should be in close contact with the inner wall of the container or slightly dried out.

5.3.2 Mycelial growth rate Incubate on sawdust medium (prepared according to A.2) at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, and the medium will be fully grown within 45 days.

5.3.3 Strain Viability Inoculate on sawdust medium, and allow the germination time of the strain to not exceed 72 hours at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

5.4 Liquid bacterial cultures

5.4.1 Sensory Requirements It should comply with the requirements in Table 5.

5.4.2 Mycelial volume ratio After the bacterial solution was allowed to stand for 30 minutes, the mycelial volume ratio was greater than or equal to 70%.

5.4.3 Fermentation Termination pH When fermentation is terminated, the pH of the liquid culture is 4.5-6.6.

5.4.4 Strain Viability Mycelial balls were inoculated on PDA medium, and the germination time of the strain was no more than 24 hours at $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$.

6 Test Methods

6.1 Container Inspection Visual inspection under natural light.

6.2 Species testing The seed supplier confirmed the seed quality was qualified through fruiting tests in accordance with the requirements of NY/T 2588.

6.3 Biological testing

6.3.1 Microscopic Examination A small amount of mycelium can be directly picked from the solid microbial culture sample to make a water seal slide; or a liquid microbial culture solution can be directly aspirated to make a water seal slide. Alternatively, mycelial balls can be inoculated onto PDA medium under aseptic conditions and cultured at $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 4-7 days. A small amount of mycelium can then be collected and processed into a solution. Cover the film. Table 2 shows the observation of mycelial growth, clamp connections, and presence of contaminants using an optical microscope with a magnification of at least 10x40. The water seal plate should be observed, and no less than 50 fields of view should be observed for each sample.

6.3.2 Mold Test Under aseptic conditions, take a small amount of culture suspected of being contaminated with mold and inoculate it onto a PDA medium slant or plate. A case is left uninoculated. PDA culture dishes served as blank controls, while PDA culture dishes inoculated with normal mycelia served as negative controls. Incubation was carried out at $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 5~7 days, and colonies were observed. If the mycelium is discolored and different from that of black fungus, or has an unusual odor, or produces a large number of conidia, it is all due to mold contamination.

6.3.3 Yeast test Under aseptic conditions, take a small amount of culture suspected of being contaminated with yeast, and set up an uninoculated PDA culture dish as a blank control. Follow aseptic procedures. Inoculate into PDA medium and incubate at $25^{\circ}\text{C}\sim 28^{\circ}\text{C}$ for 3~5 days. Colonies without filamentous structures or with an unusual odor are likely yeast. pollute.

6.3.4 Bacterial testing Under aseptic conditions, take a small amount of culture suspected of bacterial contamination and inoculate it into a container of 10 mL of nutrient broth (prepared according to A.4). In the test tubes, a blank control of uninoculated nutrient broth culture medium was also included. The cultures were incubated at 25°C~28°C with shaking for 1~2 days, and the culture results were observed. Check if the liquid is cloudy. Cloudiness indicates bacterial contamination, while clarity indicates no bacterial contamination.

6.3.5 Examination of insects and their eggs The test shall be performed according to the method described in NY/T 1284.

6.4 Sensory inspection Visually inspect the amount of mother culture medium poured in under natural light, the mycelial growth status, mycelial characteristics, colony characteristics, culture medium, antagonistic phenomena, and so on. Sensory requirements include angular change, ear buds, bacterial suspension state, contaminating colonies, and mycelial characteristics. If necessary, use a 5x magnifying glass to observe contaminating colonies and mycelial balls. feature.

6.5 Measurement of mycelial growth rate

6.5.1 Mother Seed Take five 90mm diameter petri dishes, pour in PDA medium prepared according to A.1, and after cooling, inoculate with mother culture mycelial blocks of the same size. After incubating the culture dish at 25°C±1°C for 7 days, the diameter of the colonies in each dish was measured and the average growth rate was calculated.

6.5.2 Original species and cultivated species Take 10 plastic bags (17cm x 35cm), prepare sawdust culture medium according to A.2, fill each bag with an equal amount of culture medium to make a mushroom bag, sterilize and cool. Then, an equal amount of original or cultivated spawn was inoculated into the culture bags and cultured at 25°C±1°C. The number of days it took for the mycelium to fully colonize the culture medium was recorded, and the average number of days to colonize the medium was calculated. Number of days.

6.6 Observation of bacterial strain viability The mother culture or liquid culture of the bacterial strain to be tested is inoculated into PDA medium, with at least 5 mycelial blocks of the same size inoculated into each petri dish. Mycelial balls were inoculated into three petri dishes for each sample and cultured at 25°C±1°C. The mycelial blocks or mycelia were observed and recorded visually every 12 hours. The time required for bulb germination was observed continuously for 3 days. The original and cultivated strains to be tested were inoculated into sawdust culture medium, with 5 bottles (bags) inoculated for each strain. The inoculation amount was consistent in replicates. Cultivate at 25°C±1°C, observe the germination time of the strain with the naked eye every day, observe continuously for 3 days and record the germination time.

6.7 pH Detection of Liquid Bacterial Cultures Under aseptic conditions, 30 mL of liquid