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Microbial inoculants in agriculture

农用微生物菌剂

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Microbial inoculants in agriculture

1 Scope

This standard specifies the terms and definitions, product classification, requirements, test methods, inspection rules, packaging, marking, transportation, storage of microbial inoculants in agriculture (i.e., microbial inoculants).

This standard applies to microbial inoculum products in agriculture.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Standard; however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 1250-1989 Rules for expression and judgement of limiting values

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

GB 18877-2002 Organic-inorganic compound fertilizers

GB/T 19524.1 Determination of fecal coliforms in fertilizers

GB/T 19524.2 Determination of mortality of ascarid egg in fertilizers

QB/T 1803-1993 General methods of determination for industrial enzymes

3 Terms and definitions

The following terms and definitions apply to this standard.

3.1

Microbial inoculants in agriculture

The live bacteria preparations, which are processed from target

If the dilution method [Most Probable Number (MPN) 5 pipe method] is used, it is performed in accordance with the provisions of Appendix C.

6.3.2.1 Serial dilution

Weigh 10 g of the sample (accurate to 0.01 g). Add 100 mL of sterile water (or physiological saline), which contains glass beads [for liquid inoculants, take 10.0 mL AND add 90 mL of sterile water (or physiological saline)]. Let it stand for 20 min. Fully shake it on a rotary shaker at 200 r/min for 30 min, to form a mother liquor bacterial suspension (basic liquid).

Use a sterile pipette to pipette 5.0 mL of the above-mentioned mother liquor bacterial suspension. Add 45 mL of sterile water (or physiological saline). Perform serial dilutions according to $1 \rightarrow 10$, to respectively obtain the bacterial suspension of the dilution $1 \rightarrow 1 \times 10^1$, $1 \rightarrow 1 \times 10^2$, $1 \rightarrow 1 \times 10^3$, $1 \rightarrow 1 \times 10^4$... (the sterile pipette shall be replaced for each dilution).

6.3.2.2 Sample addition and cultivation

Take 3 consecutive suitable dilutions for each sample. Use a sterile pipette to respectively pipette 0.1 mL of the bacterial suspension of different dilutions. Add them to the pre-prepared solid medium plate. Respectively use a sterile glass spatula, to uniformly coat the bacterial suspension, of different dilutions, on the surface of the agar.

Each dilution is repeated 3 times. At the same time, use the sterile water (or physiological saline) as a blank control. Culture it under appropriate conditions.

6.3.2.3 Colony identification

According to the technical data of the tested bacteria, for each dilution, take different types of representative colonies, to confirm the effective bacteria, through technical means such as smears, staining, microscopy. When the count of colonies appears in the blank control cultural dish, the test result is invalid. The test shall be repeated.

6.3.2.4 Colony count

Take the dilution plate, which appears 20 ~ 300 colonies, as the counting criteria (10 ~ 150 colonies for filamentous fungi). Respectively count the number of effective viable bacteria and the number of miscellaneous bacteria. When there is only one dilution AND the average number of colonies is between 20 ~ 300, the average number of colonies is used for calculation. If there are two dilutions, when the average count of colonies is between 20 ~ 300, it shall be determined, according to the ratio of the total number of the two colonies. If the ratio is less than or equal to 2, it shall calculate the average number of the two. If it is greater than 2, the average number of colonies, which have smaller dilution, shall be

instrument reading is stable.

6.3.7.3 Particle samples

The sample is first crushed and pass through a 1.0 mm test sieve. Make measurement, according to the method of 6.3.7.2.

6.3.8 Determination of the number of fecal coliforms

It shall meet the requirements of GB/T 19524.1.

6.3.9 Determination of the mortality of ascaris eggs

It shall meet the requirements of GB/T 19524.2.

6.3.10 Determination of cellulase activity and protease activity

It shall meet the requirements of Appendix D.

6.3.11 Determination of arsenic, cadmium, lead, chromium, mercury

It shall meet the requirements of 5.12 ~ 5.17 in GB 18877-2002.

6.3.12 Inspection of shelf life

Before the shelf life indicated in the product manual, measure the corresponding indicators of the product, according to the methods 6.3.1 ~ 6.3.11.

7 Inspection rules

The digital rounding of product technical index in this standard shall comply with the provisions of GB 8170. The qualification judgment of product quality shall comply with the provisions of the comparison method of rounding values in GB 1250-1989.

7.1 Sampling

The products, which are processed from each fermenter's bacteria liquid (or each batch of solid fermentation), as a batch, to carry out sampling inspection. During the sampling process, it shall strictly avoid the contamination of bacteria.

7.1.1 Sampling tools

Sterile plastic bags (bottles), metal spoons, samplers, measuring cylinders, kraft paper bags, glue, sampling seals, sampling sheets, etc.

7.3 Judgment rules

7.3.1 Any products, which meet the requirements of one of the following clauses, are qualified products

- a) Products whose technical index meet the requirements of the standard;
- b) In the product appearance, moisture, fineness, pH value and other test items, if there is 1 item that does not meet the requirements, BUT the other technical index meet the requirements.

7.3.2 Any products, which meet the requirements of one of the following clauses, are unqualified products

- a) The number of effective viable bacteria does not meet the technical indicators;
- b) The number of molds and miscellaneous bacteria does not meet the technical indicators;
- c) The miscellaneous bacteria rate does not meet the technical indicators;
- d) The fecal coliforms do not meet the technical indicators;
- e) The mortality of ascaris eggs does not meet the technical indicators;
- f) Any content of arsenic, cadmium, lead, chromium, mercury does not meet the technical indicators;
- g) The enzyme activity, which is measured in the organic material decomposing agent product, does not meet the technical indicators;
- h) In the inspection items of appearance, moisture, fineness, pH value, etc., there are more than 2 items (inclusive) that do not meet the requirements.

8 Packaging, marking, transportation, storage

8.1 Packaging

Choose appropriate packaging materials, containers, forms, methods, according to different product dosage forms, to meet the basic requirements of product packaging.

The product packaging shall contain the product qualification and instructions for use. In the instruction for use, it shall indicate the scope of use, methods,

dosage, precautions.

8.2 Markings

The content, which are marked on the label, shall comply with the provisions of national laws and regulations.

8.2.1 Product name and trademark

It shall indicate the general name of the product, that has been specified by the national standards and industry standards. For the name of the product OR the name of the product with a special purpose, it may be marked, under the general name of the product, by the font of smaller size.

If the national standards and industry standards do not provide for the general name of the product, it shall use the name of the product, that will not cause misunderstanding and confusion among users and consumers.

Enterprises can mark the registered trademarks.

8.2.2 Product specifications

It shall indicate the net weight of the product in each package; use the national legal unit of measurement. The error range of the marked net weight shall not exceed $\pm 5\%$ of its stated value.

8.2.3 Implemented standard of product

It shall indicate the standard number implemented by the product.

8.2.4 Registration certificate number of product

It shall indicate the valid registration certificate number of product.

8.2.5 Name and address of manufacturer

It shall indicate the name, address, postal code, contact number of the manufacturer, who has been registered in accordance with the law AND can bear the responsibility for product quality. For the imported products, it may be exempted from the marking of the name and address of the manufacturer; however, it shall indicate the place of origin (country/region) of the product, as well as the name and address of the agent or importer or seller, which is legally registered in China.

8.2.6 Date of production or production batch number

It shall indicate the production date or production batch number of the product,

Appendix C

(Normative)

Dilution method (MPN 5-tube method)

C.1 Dilution

Weigh 10.0 g of the sample. Add 100 mL of sterile water, which contains glass beads (for liquid inoculants, take 10.0 mL AND add it into 90 mL of sterile water). Let it stand for 20 min. Shake it on a rotary shaker at 200 r/min for 30 min, to obtain a bacterial suspension, which has a dilution of 1×10^1 .

Use a sterile pipette, to pipette 5.0 mL of the above bacterial suspension into a triangular flask, which contains 45 mL of sterile water. Shake well, to obtain the bacterial suspension of 1×10^2 dilution. According to this method, prepare the bacterial suspension of dilution 1×10^3 , 1×10^4 , 1×10^5 ... (the sterile pipette shall be replaced for each dilution).

C.2 Sample addition

Choose the appropriate 5 serial dilutions. Use a sterile pipette, to pipette 1.0 mL of the bacterial suspension of different dilutions. Add them to the prepared threaded-opening test tube, which contains 9.0 mL of sterile medium. For each dilution, repeatedly inoculate 5 test tubes (replace the sterile pipette between different dilutions). Use the sterile culture medium as a control, at the same time.

C.3 Culture

Tighten the plastic cap AND shake well immediately after inoculation. Place the inoculated test tube, for culture, under suitable conditions.

C.4 Calculation

According to whether there is growth of the microorganism to be tested OR the positive or negative physiological response in each dilution series test tube, obtain the quantitative index. Find the approximate value, from the corresponding MPN statistical table (see Table C.1); then, it can calculate the number of effective viable bacteria in the test sample, which is expressed in one million pieces/mL or one million pieces/g.

Calculation method:

The number of effective viable bacteria in a 1 mL (or 1 g) of sample = the approximate value of the number of bacteria x the dilution factor of the first digit of the quantity index

Appendix D

(Normative)

Determination of enzyme activity

D.1 Determination of cellulase activity

D.1.1 Principle

Using Carboxyl methyl cellulose (CMC) as a substrate, to generate reducing sugars, after cellulase hydrolysis. The 3,5-dinitrosalicylic acid (DNS) is an oxidizing agent, that can react with reducing sugars, to reduce the nitro group into an amino group; then the solution becomes orange; the depth of the orange is proportional to the concentration of reducing sugar. Therefore, the colorimetric method can be used, to obtain the content of reducing sugars; then calculate the size of cellulase activity, from the number of reducing sugars.

D.1.2 Reagents and solutions

- a) Sodium phosphate buffer (0.2 mol/L, pH 6.0): Mix 12.3 mL of 0.2 mol/L disodium hydrogen phosphate (Na_2HPO_4) and 87.7 mL of 0.2 mol/L sodium dihydrogen phosphate (NaH_2PO_4).
- b) CMC buffer: Accurately weigh 0.625 g of carboxyl methyl cellulose. Dissolve it in 100.0 mL of sodium phosphate buffer. Heat and stir to dissolve it.
- c) DNS developer: Weigh 10.0 g of 3,5-dinitrosalicylic acid. Dissolve it in distilled water. Add 20.0 g of sodium hydroxide, 200.0 g of potassium sodium tartrate, 500.0 mL of water. Heat to dissolve it. Add 2.0 g of re-steamed phenol, 0.5 g of anhydrous sodium sulfite. Cool down after all dissolving. Make its volume reach to 1000.0 mL.
- d) 1000 $\mu\text{g/mL}$ standard glucose solution: Accurately weigh 25.0 g of glucose. Use distilled water to make its volume reach to 25.0 mL.

D.1.3 Instrument and equipment

- a) Spectrophotometer;
- b) Electronic balance;
- c) Oscillator;
- d) Incubator.

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