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Determination of Antibacterial Activity for Microbial Secondary Metabolites - Inhibition Zone Method

微生物源抗生素类次生代谢产物抗细菌活性测定 抑菌圈法

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Table of Contents

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1 Scope

This Standard specifies the method for determination of the antibacterial activity for secondary metabolites of antibiotics derived from microorganisms by the inhibition zone method.

This Standard is applicable to the determination of antibacterial activity of secondary metabolites of antibiotics derived from microorganisms.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

GB 19489 Laboratories - General Requirements for Biosafety

3 Terms and Definitions

For the purpose of this document, the following terms and definitions apply.

3.1 Median inhibitory concentration; IC_{50}

The concentration at which the inhibition rate of bacteria reaches 50%.

3.2 Antibacterial activity

The ability to inhibit the growth and reproduction of bacteria.

3.3 Inhibition zone

The circular zone of aseptic propagation at the boundary between the surface of the solid medium and the specimen.

7.4 Inhibition zone measuring instrument: accuracy of 0.1mm.

7.5 Spectrophotometer: detection wavelength of 600nm.

7.6 Sterile conical flask: capacity of 100mL and 250mL.

7.7 Sterile pipette: 1mL (with a scale of 0.01mL), 10mL (with a scale of 0.1mL) or a micropipette.

8 Operation Procedures

8.1 Strain activation

The strain was inoculated into a test tube containing 2mL of LB liquid medium; and incubate at 37°C±1°C for 12h~18h. Use an inoculating loop to pick the bacterial suspension and inoculate it on the LB solid plate by streaking; incubate in a constant temperature incubator at 37°C±1°C for 18h~24h; and then pick a single colony from the plate and inoculate it in the slope of the LB solid medium test tube; incubate in a constant temperature incubator at 37°C±1°C for 18h~24h; then store the inclined surface of the test tube in a refrigerator at 1°C~4°C as preservation bacteria. The storage period is no more than one month, and the passage shall be carried out once per month. The number of passages shall not exceed 10 generations.

8.2 Preparation of bacterial suspension

8.2.1 Use an inoculating loop to take the preservation bacteria; inoculate them on the LB solid plate by streaking; and incubate at 37°C ± 1°C for 24h.

NOTE: LB solid plates are stored at 1°C~4°C and used within 1 week.

8.2.2 Take 20mL of LB liquid medium and add it to a sterile conical flask with a capacity of 100mL. Use an inoculating loop to take a single colony on the plate in 8.2.1 and inoculate it in the LB liquid medium. Incubate at 37°C±1°C for 12h~18h.

8.2.3 The bacteria concentration adjusted the incubated by LB liquid medium to 1×10^8 CFU/mL~5×10^8 CFU/mL and use this as the test bacteria suspension. Use a spectrophotometer to determine the OD_{600} of the test bacteria suspension to be 0.5~0.65 or (other) appropriate methods to determine the concentration of the bacteria suspension.

NOTE: The test bacteria suspension is stored at 1°C~4°C and shall be used within 4h.

8.3 Preparation of tested samples

The polar sample is directly dissolved in water (the non-polar sample is fully dissolved by adding a certain concentration of surfactant); prepare it into a certain concentration

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