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Determination of Antifungal Activity for Microbial Secondary Metabolites - Mycelial Growth Rate Method

微生物源抗生素类次生代谢产物抗真菌活性测定 菌丝生长速率法

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1 Scope

This Standard specifies a method for determining the antifungal activity of secondary metabolites of antibiotics derived from microorganisms by the mycelial growth rate method.

This Standard applies to the determination of antifungal 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

3 Terms and Definitions

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

3.1 Median inhibitory concentration; IC_{50}

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

3.2 Antifungal activity

The ability to resist the growth and reproduction of fungi.

4 Principal

Mix the tested secondary metabolites with the culture medium; use the growth rate of the colonies on the culture medium to measure the ability of the secondary metabolites to inhibit filamentous fungi, and judge the activity by calculating IC_{50}.

7.2 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 of mother liquor; filter through a sterile 0.45μm filter membrane. And then use the sterile water (or the corresponding surfactant) to dilute step by step by 2 times or 5 times concentration to prepare at least 5 different concentrations of the tested solution. When diluting step by step, ensure that there is concentration point with the inhibition rate greater than 50% distribution, and there is also concentration point with the inhibition rate less than 50% distribution; prepare immediately before use.

7.3 Preparation of test plate

Respectively dilute the tested samples prepared in 7.2 with the PDA medium cooled to 55°C±1°C at a ratio of 1:9; mix well and transfer 10 mL each with a sterile pipette to a sterile petri dish; gently shake the petri dish to make it evenly flat. After it has solidified, it shall be made into a test plate. The plate prepared by mixing the corresponding solvent and PDA is used as a blank control plate for later-use.

7.4 Determination of antibacterial activity

Use a sterile punch (inner diameter of 5mm±0.1mm) to punch holes in the same radius edge of the activated indicator bacteria plate; and use sterile forceps to take the bacterial cake and place it in the middle of the detection plate prepared in 7.3. Each treatment is repeated for 5 times. Place the plate in a constant temperature incubator at 28°C±1°C for 24h~48h, take it out; and measure the colony diameter by the cross method. The average value of two measurements is the colony diameter.

8 Calculation of the Results

The inhibition rate is calculated according to Formula (1):

$$I = \frac{(D_1 - 5) - (D_2 - 5)}{D_1 - 5} \times 100\% \qquad \cdots\cdots (1)$$

Where:

I – inhibition rate;

D_1 – diameter of colony formed in the blank control plate, in mm;

D_2 – diameter of colony formed in the tested secondary metabolite plate, in mm.

Take the average of five parallel samples as the final result, and keep the calculated result to two digits after the decimal point.

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