GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 07.080

A 21

GB/T 38568-2020

Determination of growth phenotype for industrialmicrobial strain - Microdroplet turbidity

工业微生物菌株生长表型测定 微液滴浊度法

Issued on: March 31, 2020 **Implemented on: March 31, 2020**

Issued by: State Administration for Market Regulation;
Standardization Administration of the People's Republic of China.

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Determination of growth phenotype for industrialmicrobial strain - Microdroplet turbidity

1 Scope

This Standard specifies the method for the determination of growth phenotype for industrialmicrobial strain by microdroplet turbidity.

This Standard applies to the determination of the growth phenotype of bacteria, fungi and microalgae strains for industrial fermentation.

2 Normative references

The following documents are indispensable for the application of this document. For dated references, only the dated version applies to this document. For undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682, Water for analytical laboratory use - Specification and test methods

GB/T 14926.43, Laboratory animal - Bacteriological monitoring - Staining, media and reagents

3 Terms and definitions

The following terms and definitions are applicable to this document.

3.1 Reference strain

The identified stock strain, whose fermentation performance is clear, for fermentation production.

4 Principle

Generate single-cell-encapsulated droplets by the micro-fluidic chip; culture it. Calculate the growth rate according to the area of the bacteria in the droplets.

5 Reagents or materials

Unless otherwise specified, all the reagents are analytical reagents.

7 Determination steps

7.1 Strain activation

7.1.1 Prepare solid plates and liquid culture media corresponding to industrial microorganisms according to the methods that are given in GB/T 14926.43. Use a sterile inoculating loop to dip the reference strain and the strain preservation bacteria solution of the to-be-evaluated strain respectively; draw a line on the solid plate; select the corresponding temperature and other conditions to perform cultivation according to the production process parameters, until a single colony grows; respectively pick 3 reference strains and 3 single colonies of to-be-evaluated strains; inoculate them into a test tube that contains 5 mL of liquid medium; select the corresponding temperature and speed according to the production process parameters; cultivate to the logarithmic phase.

7.1.2 Pick a monoclone of the microorganism strain from the activated plate petri dish; place it in a container of 5 mL of liquid medium; select the corresponding temperature and speed according to the production process parameters; cultivate to the stable period. After removing the supernatant, add the PBS solution and repeat the washing 3 times.

7.2 Selection of microbial liquid concentration

Use the basal medium to dilute to obtain a cell suspension concentration of about 6×10^5 CFU/mL.

7.3 Preparation of agarose solution

Use the analytical balance to weigh 0.02 g of ultra-low melting point agarose; place it in a sterile EP tube; add 1 mL of sterilized basal medium to it; heat to dissolve; prepare it into a 2% (mass concentration) solution. Use a 0.22 μm sterile filter to filter while it is hot; prepare it when necessary.

7.4 Single cell droplet generation

7.4.1 Respectively take 500 μL of the microbial liquid and the agarose solution in equal volumes and mix them.

7.4.2 Take a chip; respectively add 200 μL of fluorocarbon oil to the oil phase inlet of the chip and 100 μL of low melting point agarose to the water phase inlet; connect the syringe to the chip outlet.

7.4.3 Place the chip on the hot plate (temperature stability of ±1°C, range at 30 °C ~ 37 °C); fix the plunger of the syringe at the 2 mL mark; let the liquid enter the chip through the negative pressure in the syringe; start to generate droplets. It takes about 2 minutes to generate droplets.

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