Polysaccharide content determination

1. Sample treatment:

① Strain treatment

Strain: type DH5 α

Culture method: LB medium

The culture time was 24 hours

After the culture, the absorbance (OD600) was measured at 600 nm, and the number of bacteria was calculated

After centrifugation, 10ml was added into the centrifuge tube and centrifuged at 4 ℃ and 3500rpm for 15 minutes. After that, the supernatant was separated from the precipitate. The precipitate was weighed. 30ml of 95% ethanol was added into the supernatant and cooled in a 4 degree refrigerator for 12 hours. After cooling, centrifugation was performed for 15 minutes under the same conditions. After centrifugation, the supernatant was discarded and 10ml of water was taken to dissolve the precipitate to obtain the polysaccharide solution of the bacteria to be tested

② Chemical treatment

Experimental drugs: glucose monohydrate (AR), anthrone (AR), sulfuric acid (98%)

Methods:

Weigh 0.1000g glucose monohydrate, dissolve in 100ml water, and set aside at constant volume.

Weigh 20ml water and add 80ml sulfuric acid to it to prepare sulfuric acid with concentration of 80%. 0.1000g anthrone is weighed and dissolved in 80% sulfuric acid to obtain anthrone sulfuric acid solution.

2. The experimental methods were as follows

(1) Draw the standard curve of glucose Colorimetry (OD620)

① 0.0ml, 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml of glucose solution were added into 6 test tubes, and 1.0ml, 0.8ml, 0.6ml, 0.4ml, 0.2ml, 0.0ml of distilled water were added in turn.

② 0 ml of anthrone sulfuric acid solution was added to each tube, and the mixture was homogenous.

③ The test tube was immersed in 95 ℃ water bath for 10 minutes, and the solution was kept in a uniform state at all times.

④ After the color of the solution is stable, colorimetry is carried out at 620nm with the enzyme labeled instrument.

⑤ Three parallel experiments were conducted

(2) Determination of polysaccharide solution concentration

① 1.0ml polysaccharide solution of the bacteria to be tested is added into the test tube, and then 3.0ml of anthrone sulfuric acid solution is added, and the mixture is homogeneous.

② The test tube was immersed in 95 ℃ water bath for 10 minutes, and the solution was kept in a uniform state at all times.

③ After the color of the solution is stable, colorimetry is carried out at 620nm with the enzyme labeled instrument.
④ The concentration of polysaccharide was calculated by colorimetric standard curve.