BIT-iGEM2017

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Protocol for freeze - dried bacteria experiment

Background

To achieve fast and convenient detection on chip, we designed a chamber on microfluidic chip to cultivate bacteria. However, it brought new problem-the storage and transportation of bacteria. Based on the question, we came up with an idea. Make the bacteria into dry powder with the method of vacuum freeze drying. An then, when we need to use the chip, just inject some medium, recover the bacteria and it can work for you.

From room temperature to low temperature, ice crystals may generate, and then puncture bacteria cells. So we need to find a reagent to protect bacteria. At the beginning, we choose glycerol. Soon, we found glycerol is too sticky too let the powder out(Fig.1). We decided to use skimmed milk whose quality fraction is 10% to have a try.

(Fig.1 The parts surrounded by dark green circles are dry powder. The protective reagent is glycerol.)

Design

Freeze the suspension of bacteria and protective reagent at -80℃ for an hour. Open the vacuum freeze dryer and push the button of “Compressor” after 5 minutes. Freeze the cold trap of the dryer at -53℃ for an hour. And then, put the pre-frozen suspension into the cold trap. In a vacuum state, the water in suspension will sublime into water vapor leaving the system. Dry powder is left(Fig.2).

(Fig.2 The protective reagent of 3 groups on the right and left is without any treatment. The reagent of 2groups in the middle is sterilized at 115℃ for 15 minutes.)

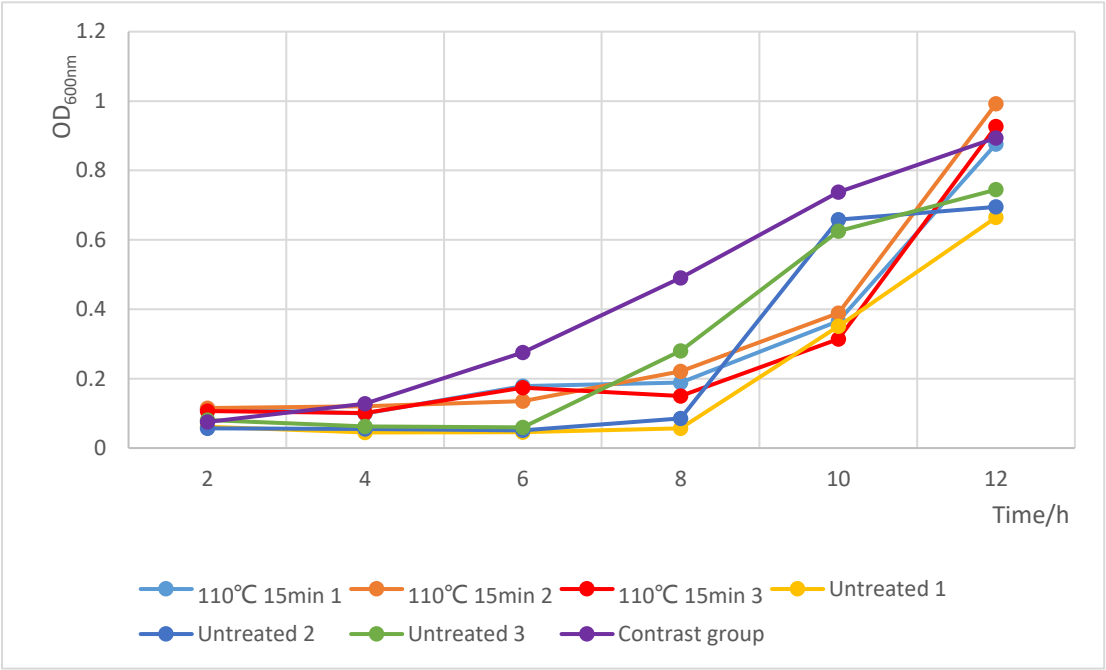
We also need an index to evaluate the effect of vacuum freeze drying on bacteria. So we designed such an experiment: we use E coli transformed into BBa_K2305004 as material. After the bacteria was made into dry powder, dissolve the powder with LB medium, resuscitate the bacteria in a shaker of 37℃ and then measure the growth curve. By comparing the curve trend before and after freeze drying, we can make the effect clear.

Results

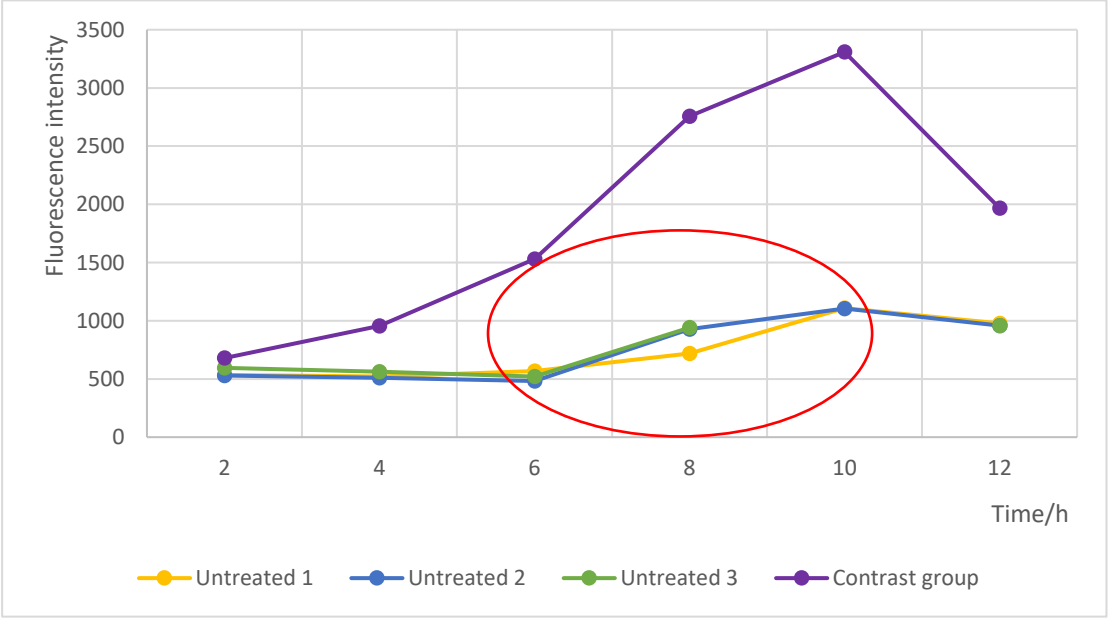
According the curves, we can include that:

(A).If the skimmed milk is sterilized at 115℃ for 15 minutes, the bacteria can grow better than those untreated(Fig.3).

(B).Though there are some differences between test groups and contrast group, they all have linear area, which we can use in our project(Fig.4). It confirms making bacteria into dry powder is feasible.



(Fig.3 The growth curves of test groups and contrast group)

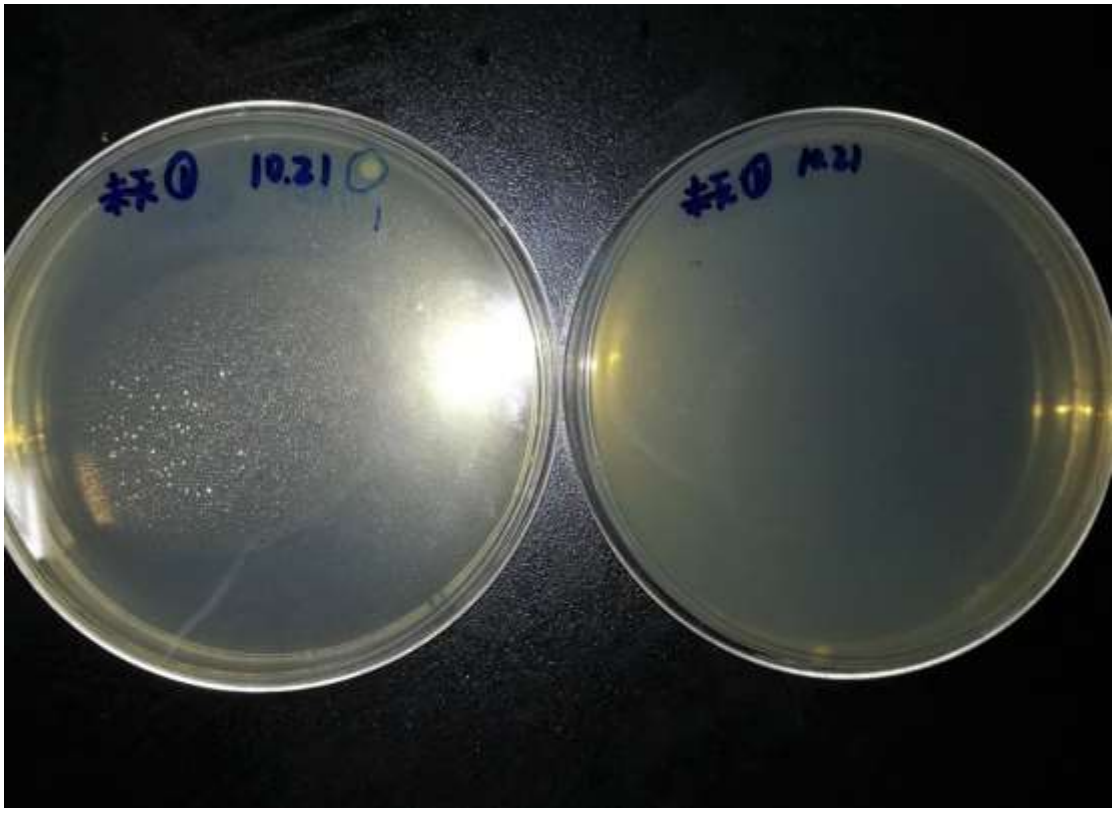


(Fig.4 The relationship curves between fluorescence intensity and time of test groups and contrast group.

The part surrounded in red circle is linear range.)

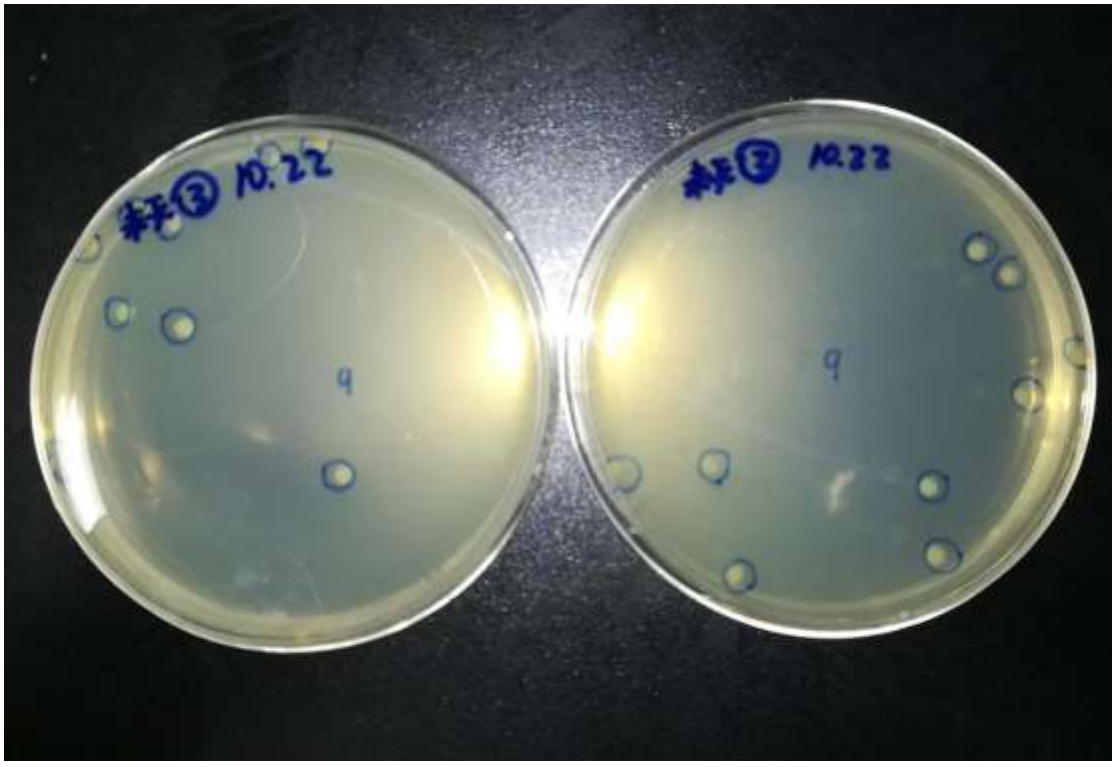
Discussion

We also used the method of dilution coating to measure the viable bacteria cells after 11 hours. But the results are not so well, perhaps the dilution factor is too big.

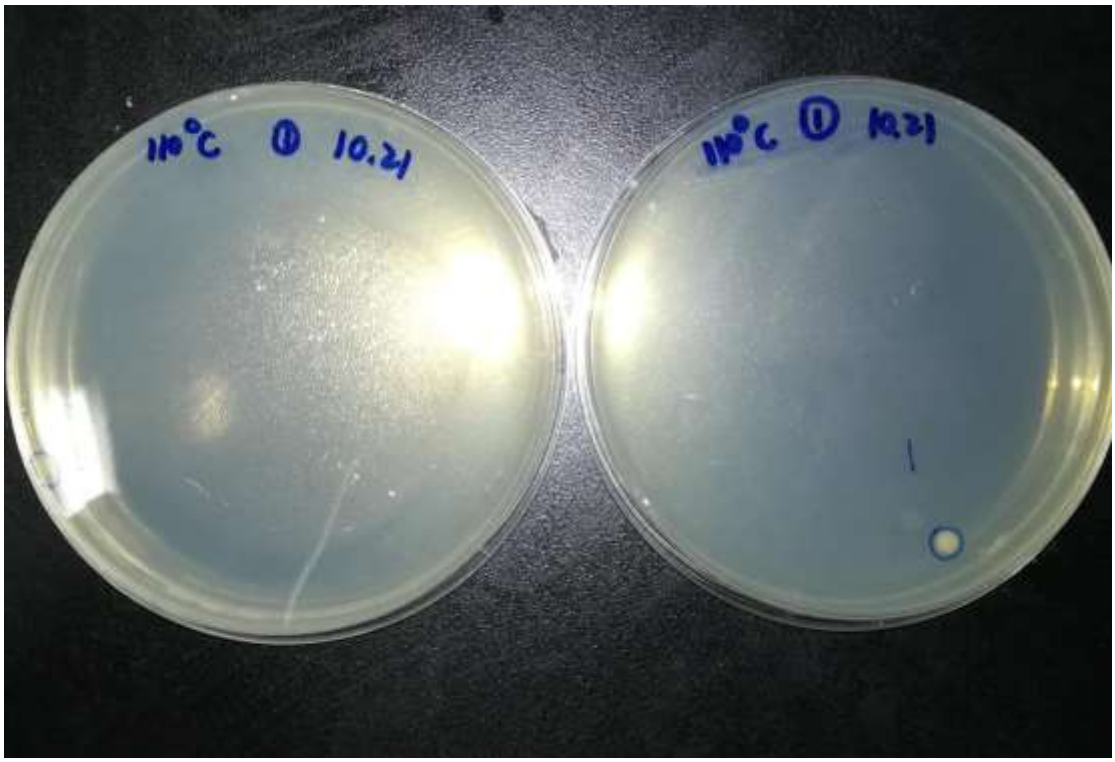


(Fig.5 Test group 1: the protective reagent was untreated. LB Solid medium plate after 48 hours.)

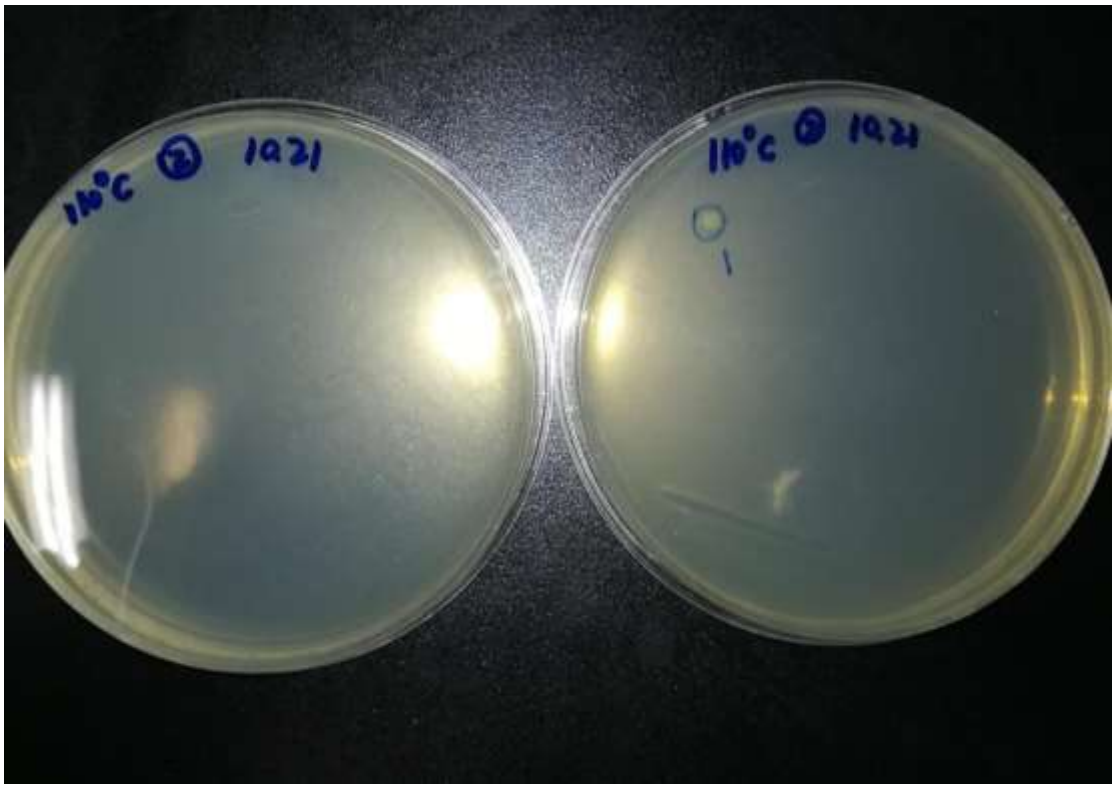
(Fig.6 Test group 1: the protective reagent was untreated. LB Solid medium plate after 48 hours.)



(Fig.7 Test group 1: the protective reagent was untreated. LB Solid medium plate after 48 hours.)

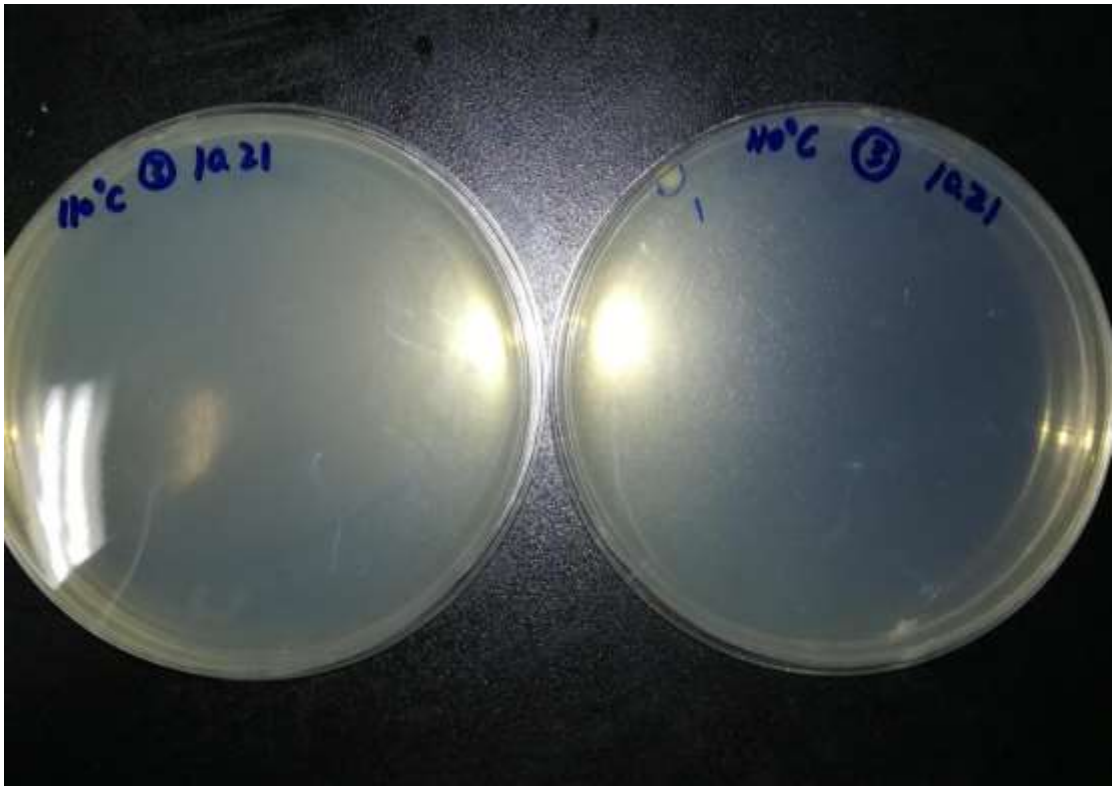


(Fig.8 Test group 2: the protective reagent was sterilized at 115℃ for 15 minutes. LB Solid medium plate after 48 hours.)

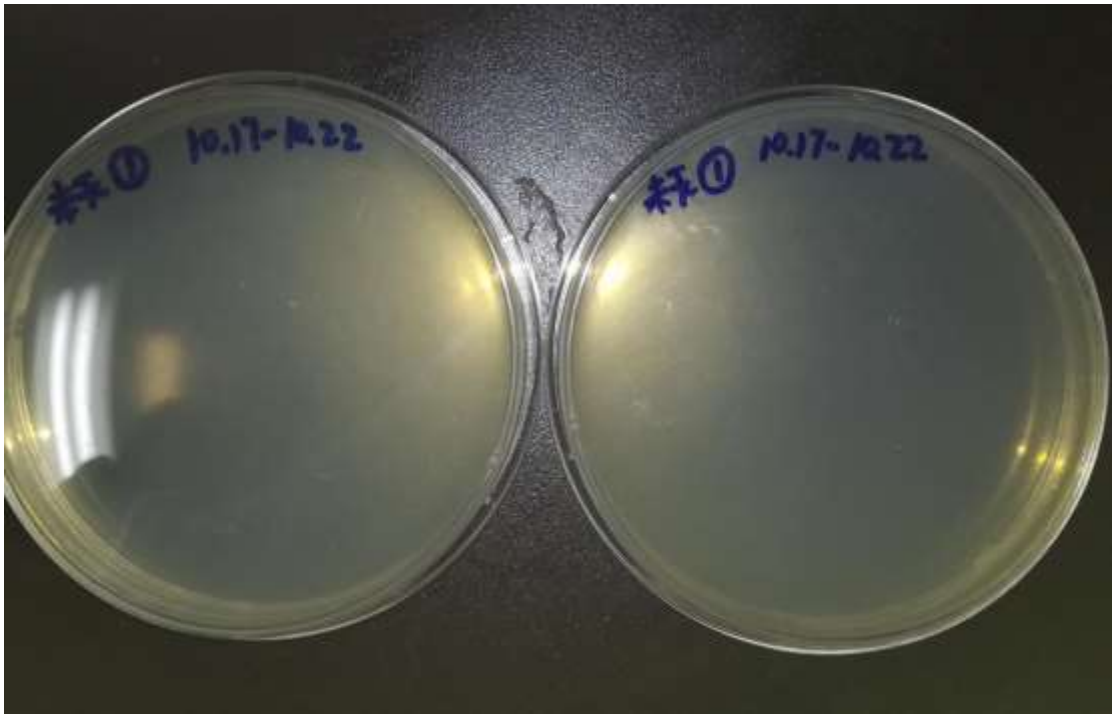


(Fig.9 Test group 2: the protective reagent was sterilized at 115℃ for 15 minutes. LB Solid medium plate

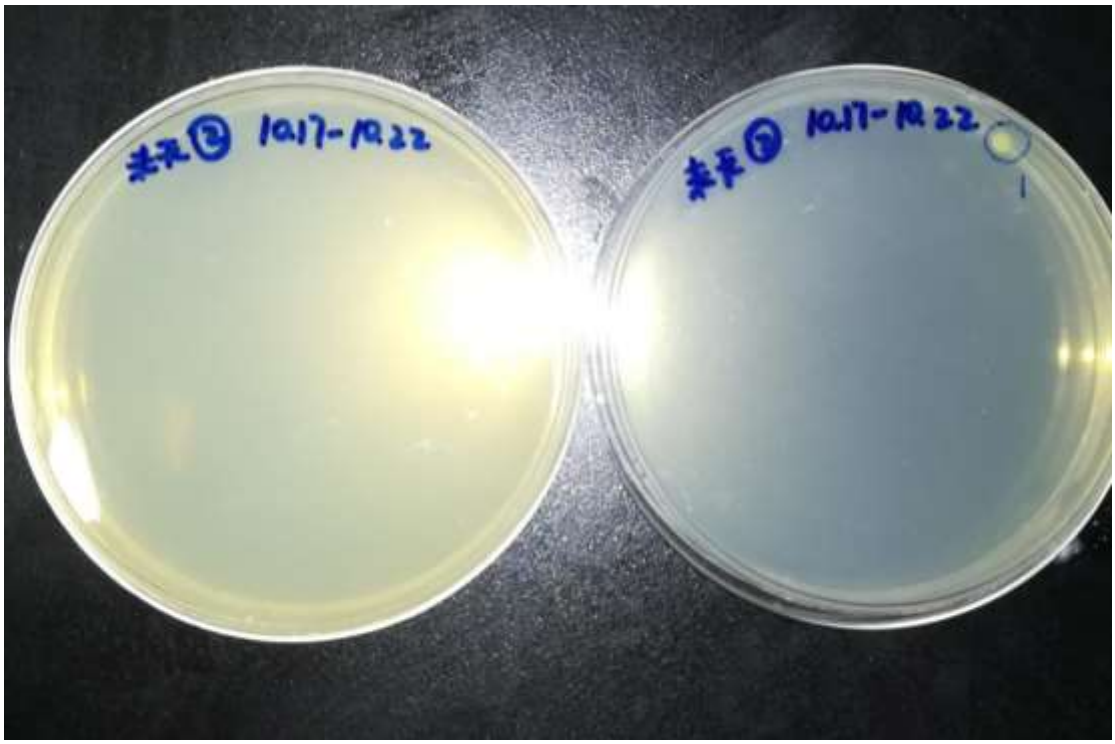
after 48 hours.)



(Fig.10 Test group 2: the protective reagent was sterilized at 115℃ for 15 minutes. LB Solid medium plate after 48 hours.)



(Fig.11 Test group 3: the protective reagent was untreated. LB Solid medium plate after 48 hours.)



(Fig.12 Test group 3: the protective reagent was untreated. LB Solid medium plate after 48 hours.)

(Fig.13 Test group 3: the protective reagent was untreated. LB Solid medium plate after 48 hours.)

Table1.

After 48 hours, we counted the colonies and recorded the average.

Test group	Parallel test	Numbers of colonies(×E5 cfu/mL)
1	1	1
	2	5
	3	9
2	1	1
	2	1
	3	1
3	1	0
	2	1
	3	1