Purpald test for butyraldehyde detection

Introduction

Purpald is a reagent generally used for formaldehyde detection. We had two bacterial strains, other transformed with a butyraldehyde-producing enzyme and other not. We wanted to compare butyraldehyde production between the two, and got the idea to try using Purpald if it could detect the difference. As there was no prior evidence of Purpald being used for butyraldehyde detection and we were running out of time, we did not actually conduct the experiment. Thus, **the protocol has not been tested**.

Thanks to Andras Pasztor for helping with planning the lysis part: how to lyse the cells, what buffers to use.

Materials

- Butyric acid
- Roche protease inhibitor tablets
- 50 mM Tris-HCl pH 7,5
- Lysozyme powder
- Purpald
- NaOH (> or = 0,5M)
- 96-well plate
- Microplate reader (550 nm)

Procedure

Butyrate buffer preparation

1. 1 ml butyric acid + 100 ml water = 0,1 M butyric acid

2. Add NaOH (1-10M) while observing pH until pH = 7

Sample preparation

3. Induce 2 ml cultures of transformant strain with IPTG in shaking at 37C for 6 hours

   Also prepare a sample of a control without the butyraldehyde producing enzyme

4. Centrifuge at 8000 rcf for 2 min and discard the supernatant

   If you want to continue later, the samples can be frozen at this point in -70C

Lysis buffer preparation

5. Dissolve Roche protease inhibitor tablet in 2 ml water to create 25X solution

   25X solution can be stored at -20C

6. Combine 5 ml 50 mM Tris-HCl pH 7,5 with 0,2 ml 25X protease inhibitor and 1-1,5 mg lysozyme to prepare the lysis buffer

Lysing the cells

7. Add 1 ml lysis buffer to each bacterial pellet, resuspend and incubate in cold room for 1 hou

8. Carefully break the cells with sonication. Avoid bubbles.

9. Centrifuge 15 min at 13000 rpm

   Meanwhile, prepare purpald (step 11)

10. Discard pellet, keep supernatant on ice

Adding butyrate and Purpald

11. Dissolve purpald at 5 mg/ml in 0,5 M NaOH

    Solution must be used within an hour!

12. Pipette 50 µl of supernatant of each sample in 6 wells

13. Add 100 µl water in 3 wells of each sample

14. Add 100 µl 0,1 M pH 7 butyrate in the remaining 3 wells of each sample

    It is uncertain whether it makes sense to incubate samples at this point

15. Add 100 µl Purpald solution to each well

    Shake/tap to ensure oxygenation

16. Measure absorbance at 550 nm

    Do a time series (0 min, 1 min, 2 min, 3 min, 5 min, 10 min, 15 min, 20 min, 30 min, more if necessary) to see if there are any differences between the strains