Protocol

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Detection of Bacterial Enzymes

Utilizing 4-Methylumbelliferyl-β-D-Galactopyranoside

Introduction

Characterization of bacteria can be achieved through various methods. One simple, rapid, and reliable method to determine the presence of specific bacterial enzymes in a strain involves the utilization of fluorescent indicators created from a reaction catalyzed by bacterial enzymes. In this reaction, a non-fluorescent substrate, such as 4-methylumbelliferyl-β-D-galactopyranoside, is used to produce a visually unique and identifiable product. Thus, this non-fluorescent substrate is considered a powerful tool in the detection and classification of bacteria. This protocol details the method for detection of enzymes based on a previously published protocol that used 4-methylumbelliferyl-β-D-galactopyranoside with *P. aeruginosa* and *E. coli*, and can be used to characterize other bacteria.

Materials

- 4-Methylumbelliferyl-β-D-Galactopyranoside (GoldBio Catalog # M-620)
- DMSO (GoldBio Catalog # D-361)
- Phosphate buffered saline, PBS (GoldBio Catalog # P-271)
- Glass rod
- Agar plate containing bacterial colonies
- Qualitative filter paper
- 0.1N NaOH
- UV lamp with a wavelength of 365 nm

Storage and Handling

- 4-Methylumbelliferyl-β-D-Galactopyranoside should be stored at -20°C and protected from light.
- This product may be shipped on blue ice and should be stored at -20°C immediately upon arrival.

Method

1. Dissolve 15µmol of 4-Methylumbelliferyl-β-D-Galactopyranoside in 0.2 ml of DMSO.

2. Dilute the solution to 10 ml with PBS at pH of 7.3.

3. Using a sterile inoculating loop, take a bacterial colony from an agar plate.

4. Rub the inoculating loop onto qualitative filter paper.

5. Cover the bacterial smear with 2 drops (30 drops per ml) of 4-Methylumbelliferyl-β-D-Galactopyranoside solution.

6. Incubate at 37°C for 10 minutes.

7. After incubation cover the smear with 2 drops (30 drops per ml) of 0.1N NaOH.

   Note: NaOH increases the fluorescence intensity and washes it away from the bacterial smear so it can be easily seen.

8. Check for fluorescence using a UV lamp with a wavelength of 365 nm.

   Note: The presence of blue fluorescence indicates a release of 4-Methylumbelliferone.

Associated Products

- 4-Methylumbelliferyl-β-D-galactopyranoside (GoldBio Catalog # M-620)
- PBS Tablets (GoldBio Catalog # P-271)
- DMSO (GoldBio Catalog # D-361)

References

Maddocks, J. L. and Greenan, M. J. (1975). A rapid method for identifying bacterial enzymes. *Journal of clinical pathology*, *28*(8), 686.a