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# Visualisation of bacteria around roots

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## ABSTRACT

This protocol is carried out to observe the growth of the bacterial biofilm along with the roots in the rhizosphere. In the paper by Bennett, R. and Lynch, J (1981), they inoculated the plant with the bacterial strain and analyzed the growth of the bacterial biofilm around roots.



R. A. BENNETT AND J. M. LYNCH. Bacterial Growth and Development in the Rhizosphere of Gnotobiotic Cereal Plants. Journal of General Microbiology.  
<https://pdfs.semanticscholar.org/cf72/4b2b6e05313ad110edc7967590b5fa34ed70.pdf>

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## PROTOCOL CITATION

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






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#### Removal of roots and bacterial counts

- 1 Carefully remove the roots from the soil.
- 2 Divide the roots into three portions: a) 0 to 2 cm from the tip; b) 2 to 4 cm from the tip; and c) 4 cm from the tip to the seed.
- 3 Root samples will have to be examined before and after washing. This is done to determine: a) the pattern and intensity of microbial colonization; b) whether the washing procedure was effective in removing the bacteria from the root surface.
- 4 Place each sample in a Erlenmeyer flask containing  **10 mL** sterile distilled water
- 5 Shake for  **00:10:00** on a wrist-action flask shaker (speed setting 7)
- 6 Prepare serial 10-fold dilutions and subsequently prepare surface colony counts on nutrient agar plates.

#### Microscopy

- 7 There are two ways in which you can observe the roots under the microscope.
  - 7.1 Directly mount roots in  **0.5 Mass / % volume** of aqueous phenol/glacial acetic acid ( 15:4, by volume). Using a microscope observe the roots.
  - 7.2 The other method is where you stain the roots by immersing them for  **00:01:00** in  **5 % volume** aqueous phenol/  **6 Mass / % volume** aqueous aniline blue/ glacial acetic acid (15:1:4, by volume). Then carefully rinse and mount in  **0.5 Mass / % volume** of aqueous phenol/glacial acetic acid ( 15:4, by volume). Observe the samples using a bright field microscope.

#### Biofilm Formation

- 8 Inoculate a plate of *Bacillus* strain (from  $-80\text{ }^{\circ}\text{C}$ ) on a BHI agar plate overnight (~  $18:00:00$ ) at  $37\text{ }^{\circ}\text{C}$ .
- 9 Inoculate  $5\text{ mL}$  BHI broth from overnight agar plate (one colony) and grow for  $04:00:00$  with shaking (~200 rpm) at  $37\text{ }^{\circ}\text{C}$ .
- 10 Inoculate 1:1,000 of broth culture (  $12\text{ }\mu\text{l}$  ) into  $12\text{ mL}$  MSgg media in petri dishes.
- 11 Let biofilm form (covered with dish lid without shaking) at  $37\text{ }^{\circ}\text{C}$  (usually 3 and 7 days). Ensure that incubator has a water container to ensure the dishes do not dry out.