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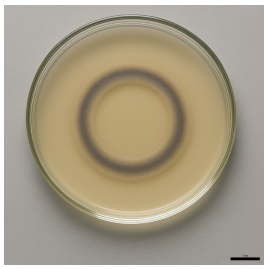
Migration of bacteria towards individual root exudate compounds



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We use this protocol and it's working.

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Abstract

This protocol was used to test the ability of seedling exudate compounds to serve as chemoattractants for *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense*. Compounds were selected from the composition of our own exudates that were produced from peas, tomatoes, and cucumbers. The concept of the semi-solid swim plate was used, where bacteria migrate towards the attractant on 0.3% agar plates. The swim medium was modified from for *R. leguminosarum* and MMAB for *A. brasilense*.

Image Attribution

The image was generated by AI

Guidelines

You will measure the diameter of the swim ring. I use a ruler and measure the outermost edge. Some of the rings can be faint, that depends on how much the bacteria will be growing while swimming. If you let them swim long enough, you may see multiple rings. We usually measure until the rings are at lest 4 or 5 cm in diameter. If you plot the ring against time, you will see a bit of a lag, then a straight line.

Materials

- **Revised RB minimal media (RB):**
  - 7.3 mM K_2HPO_4, 3 mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 1 mM $(NH_4)_2SO_4$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 0.1 mM NaCl, 0.2% mannitol, supplemented with filter sterilized 0.01 mM Na_2MoO_4, 0.001 mM $FeSO_4$, 0.2% carbon based putative chemoattractant, 1 µg/mL thiamine, 1 µg/mL biotin, 1 µg/mL pyridoxin HCl, and 10 µg/mL $CoCl_2$ after autoclaving.

- **Carbon based seedling exudate compounds (RB carbon):**
  - 7.3 mM K_2HPO_4, 3 mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 1 mM $(NH_4)2SO_4$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 0.1 mM NaCl, 1 mM carbon based putative chemoattractant, supplemented with filter sterilized 0.01 mM Na_2MoO_4, 0.001 mM $FeSO_4$, 0.2% carbon based putative chemoattractant, 1 µg/mL thiamine, 1 µg/mL biotin, 1 µg/mL pyridoxin HCl, and 10 µg/mL $CoCl_2$ after autoclaving.

- **Amino acid seedling exudate compounds (RB amino acid):**
  - 7.3 mM K_2HPO_4, 3 mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 0.1 mM NaCl, 1 mM amino acid based putative chemoattractant, supplemented with filter sterilized 0.01 mM Na_2MoO_4, 0.001 mM $FeSO_4$, 0.2% carbon based putative chemoattractant, 1 µg/mL thiamine, 1 µg/mL biotin, 1 µg/mL pyridoxin HCl, and 10 µg/mL $CoCl_2$ after autoclaving.

Safety warnings

NA

Ethics statement

No humans or animals are involved in this protocol.

Before start

Be aware of what you want to do. You can use different bacteria, but will have to look up the minimal medium for them.

Migration of bacteria towards individual root exudate compounds

1 Test the ability of bacteria to migrate towards individual root exudate compounds. Select carbon sources and amino acids that suit the biological question for your experiment.

2 Test *R. leguminosarum* bv. *viciae* 3841 on RB carbon and RB amino acid minimal swim agar. RB carbon minimal swim agar contains all components of RB minimal medium, except D-mannitol and glycine, supplemented with 1 mM of a carbon source (e.g., fructose). For the composition of RB, see materials section.

3 Supplement organic acids (*e.g.*, citric acid) with the sodium salt of the acid and determine the pH of the solution. Adjust the pH when needed.

4 RB amino acid minimal swim agar contains all components of RB minimal medium, except D-mannitol and glycine, supplemented with 1 mM of an amino acid (*e.g.*, asparagine, arginine). The agar concentration is 0.3%.

5 To make the RB minimal carbon swim plates: add all the components from the Materials section, RB carbon until NaCl. Add agar. Then autoclave. Supplement 248.75 ml of this warm solution with filter sterilized solutions of the following:
250 µl of 1 M carbon source, that gives you 1 mM in the end.
250 µl of 0.01 mg/ml $CoCl_2$
250 µl of 1 mg/ml thiamine
250 µl of 1 mg/ml biotine
250 µl of 1 mg/ml pyridoxin HCl
Pore ~20 ml into a petri dish and let sit until semi-sold. Inoculate next day with *R. leguminosarum* bv. *viciae* 3841 or other Rleg strain. You will need 100 µl of a culture to be spotted into the center of the plate.

6 To make the RB minimal amino acid swim plates: add all the components form the Materials section, RB amino acids until NaCl. Then autoclave, notice there is no ammonium sulfate. Supplement 245 ml of this warm solution with filer sterilized solutions of the following.
5 ml of 1 M amino acid, that gives you 1 mM in the end.
250 µl of 0.01 mg/ml $CoCl_2$
250 µl of 1 mg/ml thiamine
250 µl of 1 mg/ml biotine
250 µl of 1 mg/ml pyridoxin HCl
Pore ~20 ml into a petri dish and let sit until semi-solid. Inoculate next day with *R. leguminosarum* bv. *viciae* 3841 or other Rleg strain. You will need 100 µl of a culture to be spotted into the center of the plate.

7 Production of the Rleg culture: Liquid inoculant cultures were grown in RB minimal media with mannitol for 3 to 4 days. The bacteria were pelleted at 10,000 ×g for five minutes and resuspended in fresh media with no nitrogen or carbon source. Inocula were standardized to an OD_{600} of 1 or as close as possible (±0.2).

8 Construct the composition of the minimal swim agar for *A. brasilense* Sp7 similarly. Use MMAB minimal medium as a base. MMAB carbon lacks malic acid, MMAB amino acid lacks malic acid and NH_4Cl. Supplement with 1 mM carbon source for the carbon minimal swim plates and 1 mM amino acid for the amino acid minimal swim plates. Use 0.3% agar for the swim plates. For the composition of MMAB, see Table 1.

9 Production of the Abra culture: Liquid inoculant cultures were grown in TY liquid media for two to three days at 30°C. The bacteria were pelleted at 10,000 ×g for five minutes and resuspended in minimal MMAB without nitrogen or carbon source. Inocula were standardized to an OD_{600} of 1 or as close as possible (±0.2).

10 As a negative control for the *R. leguminosarum* bv. *viciae* 3841 or *A. brasilense* Sp7 experiments, produce swim plates from RB or MMAB minimal medium that do not contain the carbon or amino acid source. You can also use a mutant that does not swim.

11 Data collection: The bacteria will 'eat' up the nutrients in the center of the plate and migrate towards the gradient that they themselves produced. That means you will see a concentric ring forming around the inoculation spot. For Rleg, that can take several days. For Abra, expect about a day. Measure the diameter of the ring in regular time interval so you get 6 to 8 data points. If you plot the diameter against time, you should get a linear increase in diameter. Calculate cm or mm/h.

12 Statistical analysis. Conduct each experiment in at least 4 replicates. Present data as bar plots or box and whiskers. Perform statistical analysis with ANOVA, followed with Tukey that compared data from each carbon source or amino acid to the negative control.

Protocol references

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A. brasilense Sp7: Tarrand et al., 1987. Can. J. Microbiol. A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov.

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