

The Effect of Different Carbon Sources on Chemotaxis and Predation of a Toluene-Degrading Bacteria



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Research Objectives

- To determine whether *Pseudomonas mendocina* can metabolize other compounds in the toluene-degradation pathway
- To determine whether *P. mendocina* prefers any of these metabolites over the others
- To determine how the utilization of each of these metabolites will effect predation by protozoa

Background

Bacteria play a major role in the food web by breaking down complex organic molecules that most other organisms cannot metabolize. This ability has become extremely important in environmental cleanup projects through bioremediation¹. Studies have shown that predation by protozoa can increase the metabolic activity of a bacterial population⁴ making it an important tool to speed up bioremediation³.

Some bacteria such as *Pseudomonas mendocina* can break down extremely toxic pollutants like toluene. Toluene at some levels is toxic to protozoa² which could potentially limit the role of protozoa in an environment which contains toluene.

The pathway by which *P. mendocina* degrades toluene to the final products, H₂O and CO₂, involves several intermediate compounds (Fig. 1). All of these intermediate compounds are potential organic sources for the bacteria. Utilization of these different food sources may have effects on the physical and chemical properties of the bacteria and may effect nutritional value to predators.

Organisms

The toluene-degrading bacteria species *Pseudomonas mendocina* was received from Lillian Young's lab and cultured on Zobell's media (5:1 peptone and yeast) and 22% filtered seawater.

Two marine ciliate protozoa, *Euplates vannus* and *Cyclidium* were cultured on ASWP from stocks already existing in the lab.

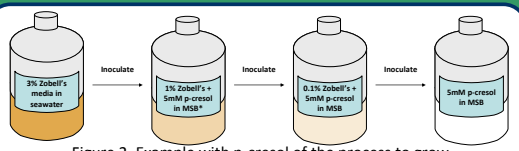


Figure 2. Example with p-cresol of the process to grow *P. mendocina* on the metabolites. *MSB is an artificial seawater solution.

Growing *P. mendocina* on the Intermediate Compounds

P. mendocina was weaned off of the Zobell's carbon source onto each of the first four intermediates in the toluene pathway (p-cresol, hydroxybenzyl alcohol, hydroxybenzaldehyde, and hydroxybenzoic acid) by gradually decreasing the concentration of Zobell's media and adding the chemical of interest as shown in Figure 2. *P. mendocina* was successfully grown on 5mM p-cresol, 0.2 g/L hydroxybenzyl alcohol, 0.5g/L hydroxybenzaldehyde, and 0.5g/L hydroxybenzoic acid.

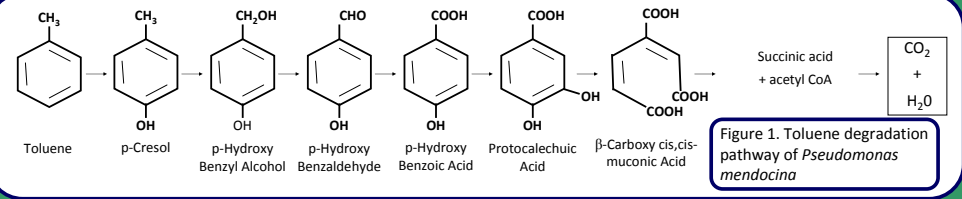


Figure 1. Toluene degradation pathway of *Pseudomonas mendocina*

Chemotactic Preferences of *P. mendocina*

Chemotaxis, the ability of bacteria to move toward a food source using chemical signals, was used as a measure of preference of the bacteria to a particular food source. Chemotaxis towards the metabolites of bacteria grown on each of the four metabolites of interest as well as a control grown on Zobell's media was measured using capillary assays (Fig. 3).

All five of the bacteria populations were chemotactic toward each of the metabolites (Fig. 4). Regardless of the media which they were grown on, the bacteria tended to move fastest toward p-cresol and faster toward the alcohol than toward the aldehyde and acid. Only *P. mendocina* grown on Zobell's moved toward MSB. The general trend of the speed of the bacteria according to which carbon source it was grown on was Zobell's > aldehyde > acid > alcohol > cresol.

Figure 3. Capillary Assay Set-up

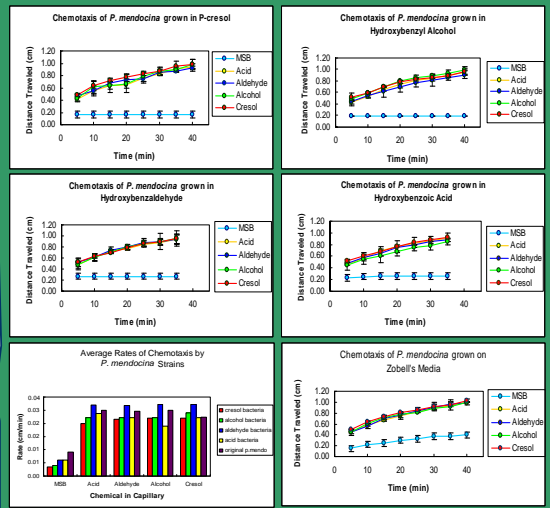
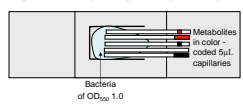


Figure 4. Capillary Assay Results for *P. mendocina* grown on different carbon sources. Bacteria were placed in the assay set up shown in Fig. 3 and the turbid bacteria dilution was observed traveling up the capillaries. The distance traveled by the bacteria was noted every 5mins. Error bars reflect the standard deviation of three replicates.

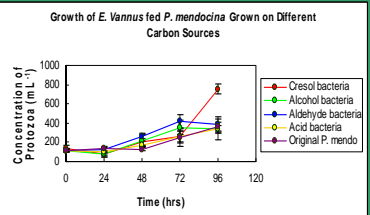


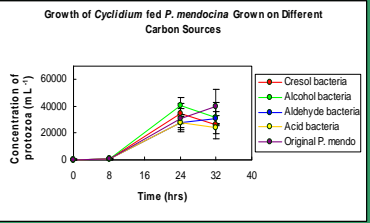
Figure 5. Predator-Prey Growth Experiment

Predator-Prey Growth Experiment

E. vannus and *Cyclidium*, the two protozoa species, were fed *P. mendocina* which had been grown on each of the metabolites. Samples of each culture were taken every 8 hrs for *Cyclidium* and every 24 hrs for *E. vannus*. Cells were killed with gluteraldehyde and counted under a microscope to monitor growth of the populations.

The *Cyclidium* populations grew substantially with all of the bacteria types within 24hrs (Fig. 5), and there was no significant difference between growth on bacteria fed one carbon source compared to the others (p= 0.05695).

E. Vannus numbers doubled in all cultures by 72 hrs. In the vials fed cresol bacteria, the population increased significantly more than the other by 96 hrs (p= 0.0008).



Discussion and Conclusions

Can *P. mendocina* metabolize the compounds in the toluene-degradation pathway?

Growth of *P. mendocina* was established on p-cresol, hydroxybenzyl alcohol, hydroxybenzaldehyde, and hydroxybenzoic acid confirming that, yes, *P. mendocina* can metabolize these compounds.

Does *P. mendocina* prefer any of these metabolites over the others?

P. mendocina showed faster rates in the chemotaxis tests toward p-cresol over the other metabolites regardless of which media it was grown on suggesting that *P. mendocina* might favor p-cresol in an environment where these sources are available. Differences in rates among the bacteria may hint at altered physiological characteristics due to carbon sources utilized.

P. mendocina which was grown on Zobell's traveled up the control capillary with MSB as if the bacteria was chemotactic towards it, although in a lesser degree than to the metabolites. Perhaps the bacteria that did not exhibit this behavior were acclimated to the metabolites and recognized them as food sources without exploring the control, while the bacteria grown on Zobell's did a little more searching moving into the MSB tube.

How does growth of *P. mendocina* on the different metabolites effect the protozoa predators?

No significant difference was measured for the growth of *Cyclidium* fed bacteria grown on any of the carbon sources. This suggests that the metabolites will have little effect on predation by *Cyclidium*.

E. vannus showed similar growth with bacteria grown on three of the toluene intermediates and bacteria grown on Zobell's, but increased growth with bacteria grown on p-cresol. This suggests that *E. vannus* populations will have higher success feeding on bacteria metabolizing p-cresol rather than other carbon sources. The cresol bacteria may be easier for *E. vannus* to ingest than the others due to altered physical characteristics like size or motility. The chemotaxis study supports the possibility of decreased motility in cresol bacteria.

References

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Acknowledgements

I would like to thank Lillian Young and her lab for providing me with the bacteria, and with ideas and advice along the way. Thank you also to Leslie Shor from Vanderbilt, who provided me with microfluidic devices to aide in my chemotaxis and predation studies, although, unfortunately, I was not able to present the data here.
 Thanks to the NSF for funding the RIOS program making this work possible.