Bloated Rhizobia: The effect of PHB storage on *Bradyrhizobium japonicum* mortality during desiccation

Kyra Underbakke College of Biological Sciences, University of Minnesota, Twin Cities
Mentors: Dr. R. Ford Denison and Dr. W.C. Ratcliff, Ecology and Evolutionary Biology

Introduction

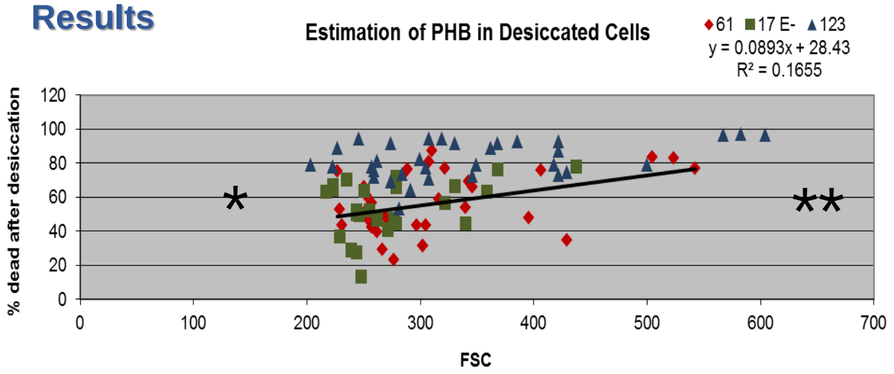
Soil bacteria known as rhizobia infect the roots of legumes, forming nodules, where they benefit the host by fixing atmospheric nitrogen into a usable form for the plant. In return, these rhizobia use plant-derived energy to reproduce, and many species accumulate large quantities (>50% cell dry weight) of the storage lipid poly-3-hydroxybutyrate (PHB). Since high PHB stores give rhizobia up to a threefold reproductive advantage over cells with low PHB during starvation conditions (Ratcliff *et al* 2008), genotypes of rhizobia that are able to escape from nodules with more PHB would be expected to increase in frequency through time. However, not all strains of rhizobia synthesize large amounts of PHB. This research investigates a potential disadvantage to storing too much PHB: that accumulating excessive amounts of PHB may lead to increased cell mortality in a drought environment.

Materials and Methods

B. japonicum were grown on soybean *(Glycine* max) for 10-40 days, then harvested by crushing nodules and extracting the rhizobia. Each nodule was diluted to $5x10^7$ cells/mL. 10 µL of this dilution ($5x10^5$ cells) was put on the lid of a 500 µL microcentrifuge tube, and air dried in a laminar flow hood for 30 min.

400 µL of standard buffer (SB) were added to each tube, and the tubes were closed and inverted to allow rhizobia to rehydrate. These tubes were vortexed for 10 sec, stained with YO-PRO-1 (live/dead stain), and run on a flow cytometer. The percent dead in each tube was calculated and regressed against the mean PHB content of each population, as estimated by mean FSC (forward scattering, a measurement of cell size).

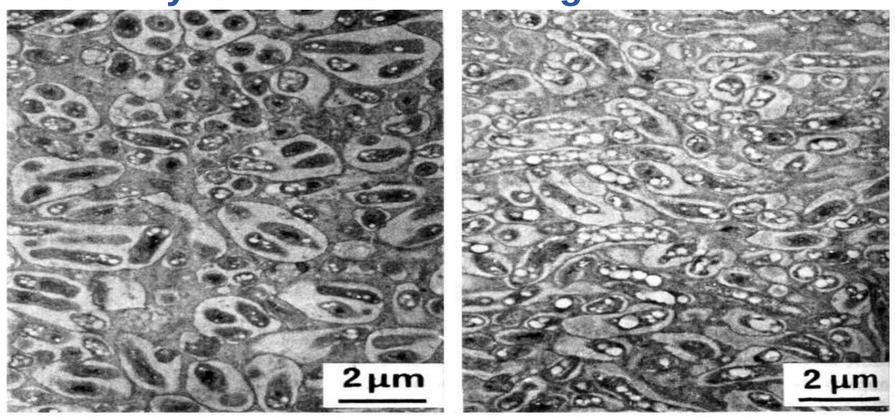
Results



*These results show a variation in the percent killed for cells with mean FSC between 200-450. This indicates that when the rhizobial cells accumulate a "normal" amount of PHB, the amount of PHB acquired is not a strong enough factor to greatly influence cell mortality during desiccation.

**However, for FSC greater than 450, the points fit the trendline more strongly. This shows that there may be a point at which PHB accumulation becomes excessive.

Bloated rhizobia: cells that hoard PHB may be less likely to survive in a drought environment



Fix(+) (wild type)

Studer & Han, 1986

Fix(-) bacteroid (does not fix nitrogen, hoards PHB)

References

Ratcliff WC, Kadam SV, & Denison RF. 2008. "Poly-3-hydroxybutyrate (PHB) supports survival and reproduction in starving rhizobia." Federation of European Microbiological Sciences (FEMS) 65, 391-399.

Hahn M & Studer D. "Competitiveness of a nif- *Bradyrhizobium japonicum* mutant against the wild type strain." FEMS 33 (1), 143-148.

Discussion

Studer and Hahn (1986) have shown that there is a trade-off for rhizobia between fixing nitrogen and accumulating. The three-fold reproductive advantage of storing PHB (Ratcliff *et* al 2008) makes it seem unlikely that low PHB-storing, nitrogen-fixing strains would survive in the wild. However, nitrogen-fixing strains persist.

The results of this research showed a **positive correlation between PHB storage and percent killed by desiccation**, indicating that **high PHB storage may not always be selected for** in rhizobial populations, despite its reproductive advantages.

Continuing Research

I am continuing the research in desiccation and cell mortality, with the following changes:

- stain with **Nile Red** (allows measurement of PHB, rather than FSC estimation)
- Four strains: USDA **123**, USDA **110**, **61**, and **17E(-)**
- Focus on **harvesting larger nodules** (as nodule size is correlated to PHB content); harvest at 30-50 days (rather than 10-40 days)
- % killed **by** desiccation (live/dead counts both before and after desiccation)