GROWTH CURVES OF R. LEGUMINOSARUM IN LIQUID CULTURE

Started liquid cultures of Rhizobium leguminosarum from plates Vinata streaked, bv. 3184 was streaked on one plate of TY + 1% agar with no selection, bv. 22 and bv. 26 were streaked on opposite sides of the same plate and were growing on TY medium + 1% agar with 50 ug/ mL kanamycin selection. 3184 had individual colonies, but 22 and 26 had grown together, so I collected a small portion of bacteria from the edge points attempting to get amounts roughly equal to the colony sizes on the wild type, 3184.

1. One liter of TY medium + $CaCl_2 \cdot H_2O$ was prepared the day before, 50 mL was aliquotted into each of 20, 250-mL flasks. The flasks were topped with a bung then covered with aluminum foil and autoclaved on the media cycle (20-minute sterilization).
2. Labeled 16, 250-mL flasks containing sterile TY medium. One was labeled "blank" and was not inoculated. The other 15 flasks were labeled with the bacteria name (3184, 22, or 26) and I, II, III, IV, or V. There were 5 replicate flasks per bacterium.
3. Sprayed and wiped down the biosafety cabinet with 70% ethanol. Transferred the flasks, the plates of bacteria, and the bacterial loop under the hood.
4. Removed the foil and bung from a flask. Placed the bung inside the foil to keep it sterile. Flamed the lip of the flask.
5. Dipped the bacterial loop in ethanol, flamed it, cooled it on an uninoculated portion of the plate, then collected a bacterial colony. Transferred the loop to the TY broth and moved it back and forth for 10 seconds to dislodge the bacteria from the loop.
6. Flamed the loop. Flamed the lip of the flask and replaced the bung and foil. Repeated this for all 15 flasks, inoculating 5 flasks with each biovar.
7. Placed the 16 flasks in the shaker at 120 rpm and 28 degrees. The actual temperature was between 30 and 31° C in the shaker as the temperature control is poor. Set the shaker at 25 degrees, but 30 was the lowest temperature the incubator could maintain.

Liquid cultures were started at 9 a.m. One mL samples were taken at 2 and 7 p.m. and read on the spec as follows:

1. Flasks were removed from the shaker and taken to the biosafety cabinet, which had been wiped down with ethanol.
2. Starting with the blank (uninoculated TY media), the foil and bung were removed as described above and the lip of the flask was flamed.
3. Using a sterile pipet tip, removed 1 mL of broth from the flask and transferred it to a disposable 1.5 mL cuvette. Flamed the lip of the flask, replaced the bung and foil, and repeated this process with each of the remaining 15 flasks. Sampled 3184, then 22, then 26.
4. Replaced the flasks in the shaker with the same conditions.
5. Using the TY as a blank, read each of the 15 samples on the spectrophotometer at 600 nm. Recorded the results.

6. Disposed of the tips and cuvettes in the biohazard waste.

DILUTION PLATING OF RHIZOBIUM LEGUMINOSARUM 26

Tube 1: Took a 1 mL aliquot out of R.l. 26, rep I. Diluted it with 9 mL of TY. 10^{-1}

Did a serial dilution as follows:

Tube 2:	1 mL tube 1 + 9 mL TY	10^{-2}
Tube 3:	1 mL tube 2 + 9 mL TY	10^{-3}
Tube 4:	1 mL tube 3 + 9 mL TY	10^{-4}
Tube 5:	1 mL tube 4 + 9 mL TY	10^{-5}
Tube 6:	1 mL tube 5 + 9 mL TY	10^{-6}
Tube 7:	1 mL tube 6 + 9 mL TY	10^{-7}

Plated out 100 uL of samples onto 6 plates – three with selection (50 ug/mL KM) and three without (TY agar). Pipetted 100 uL of 10^{-5}, 10^{-6}, and 10^{-7} onto separate plates of KM and TY. Using the bacterial loop, I spread the bacterial culture over the plate, flaming the loop between plates. Placed the plates in the 28°C incubator. This will tell me which dilution is most appropriate for counting individual bacterial colonies. Done at 10 a.m.

INOCULATION OF NEW FLASKS OF RHIZOBIUM – PURPOSE: TO PLATE OUT BACTERIA TO CORRELATE OD READING TO BACTERIAL CELL COUNT

At 4:30 p.m., I inoculated new flasks of TY medium with R.l. 3184, 22, and 26. The purpose is to repeat the growth curve experiment for verification and to take samples for spec-ing and for plating at the same time to correlate OD reading with the bacterial cell counts. Also, I added selection to the flasks containing 22 and 26 (1 mL KM stock [5 ug/mL] to 100 mL TY media). For comparison of straight growth curves, Vinata and I decided that selection would add an additional variable that could not be replicated in all three treatments. Colonies were taken from KM plates, and therefore should be true mutants.

Chose to down-size the experiment, given the time involved and the extra component of the experiment. Inoculated 3 flasks with 3184, 3 with 22, and 3 with 26. Placed these and one uninoculated flask into the shaker. Sampling will begin tomorrow morning.

2/12/02

RESULTS – RHIZOBIUM LEGUMINOSARUM GROWTH CURVES

Took the last sample from 22 and 26 at 10 a.m. this morning. Autoclaved the cultures and cleaned the flasks.

Graphed the growth curves of R.l. 3184, 22, and 26. R.l. 22 and 26 had almost identical curves. They take longer to reach log-phase growth, logarithmic growth is not as rapid as in the wild type (i.e. the slope of the line is less steep), and the OD at stationary phase is much lower than that of the wild type (3184).

SPEC READINGS AND DILUTION PLATING OF RHIZOBIUM LEGUMINOSARUM

Took samples from the second set of R.l. cultures: 3184, 22, 26, and the blank. Took 1 mL samples of each to run on the spec. Took an additional 100 uL sample from each flask and placed it in an eppendorf tube. Ran the samples on the spec. OD on 3184 was already above 1, so I diluted these samples to 10^{-6} as described on 2/11/02 (Haynes notebook 3). OD's on 22 and 26 were low or negative, so I plated these samples out with no dilution. Used 100 uL of the sample (22 and 26) or 10^{-6} dilution (3184) to inoculate plates of TY + 1% agar. Used the bacterial loop to distribute the sample evenly, flaming between. Also plated out 100 uL of TY broth as a control.