Lab Project
Enchrichment & N Cycle

Enrichment Techniques
Goal - tease target microbes from population
Inoculum - mixed sample with target microbes
Many strategies thereafter - physical, chemical
Understand inoculum, strategies - ALL projects!

Nitrogen Fixation
N2 fixed to NH3 and NH2 groups - COSTS ATP
ONLY by Prokaryotes - Bacteria and Archaea
Conserved O2-intolerant Nitrogenase/Fe-Mo
Understand Acetylene Reduction Assay

Some Nitrogen Fixer Diversity
Cyanobacteria: colonial, heterocysts resist O2
Methanogens/Archaea: live in anoxic habitats
PJ - Rhizobium: symbiotic leghemoglobin
PJ - Azotobacter: fastest ETC uses O2

Symbiotic Nitrogen Fixation
Legumes: root hairs secrete inducer molecules
Symbionts: soil Rhizobium, Proteobacteria
Separate/no fixation; together/leghemoglobin
Bacteroids: misshapen Rhizobium in nodules

Free-Living Aerobic Azotobacter
Proteobacteria - capsule-producing rods
Beijerink - first historic enrichment (Fig. 22.1)

Nitrification, Aerobic Lithotrophy
Two-step - ammonia then nitrite oxidation
NH3 + O2 yields NO2 + H2O, Nitrosomonas
NO2 + O2 yields NO3, Nitrobacter
Both are Proteobacteria; packed with lamellae

Anaerobic Nitrate Reduction
Denitrifying (to N2) OR Nitrifying (to N-fixed)
NO3 + CH2O yields N2 + CO2, Pseudomonas
NO3 + CH2O yields NO2, NH3 + CO2, Enterics

ACTIVITIES

Azotobacter - Work Individually, Incubate RT°
Two N-free enrichment flasks have been selecting for 3 weeks; everyone needs to share!
Looping from oxic TOP of enrichment, streak onto 1 N-free plate - watch for slimy colonies
Notebook records – thoroughly describe plates, target colony texture and shape

Azotobacter N-Free Media		
Solution A	*Solution B*	Prepared as liquid and agar (15-20 g/L) plates, with agar added to Solution B. Combine 1A:1B after autoclaving. This high-sugar enrichment medium is designed to enhance capsule production, a distinct Azotobacter trait. This medium is also highly selective for N2-fixers because it contains no nitrogen.
K2HPO4 : 1.6 g	MgSO4: 0.4 g	
KH2PO4: 0.4 g	CaSO4: 0.2 g	
	FeSO4/7H2O: 0.006 g	
	MoO3: 0.002 g	
	sucrose: 10 g	

Nitrate Reduction - Work In Pairs, Incubate RT°
Two 10-ml, 10-fold organic soil dilutions have been prepared; everyone needs to share!
Working in pairs, inoculate 3 nitrate reduction tubes: soil dilution, E. coli, Pseudomonas
After a week, complete all portions of the nitrate reduction test as described below
Notebook records – thoroughly describe tubes and interpret testing at each stage

Nitrate Reduction Media	Prepared as 7 ml light gold liquid tubes with Durham tubes. After growth, read in order: (1) Bubble? (yes = NO3 to N2); (2) Add 10 drops A & B. Red? (yes = NO2); (3) If not, add 10 grains Zn and wait 2-3 minutes. Red? Yes = NO3 present/no NO3 reduction; No = NH3, NH2-compounds.
Beef Extract: 3 g	
Peptone: 5 g	
KNO3: 1 g	

Rhizobium - Work In Pairs, Incubate RT°
Working in pairs, obtain 2 root nodules, lightly wash off dirt and surface treat 2 minutes, comparing…
1 nodule with bleach disinfectant; 1 nodule with alcohol antiseptic – KEEP TRACK which is which
After treatment, separately place each nodule in 1 drop sterile water - crush with sterilized forceps
PROPERLY streak each treatment onto provided Rhizobium plates – watch for slimy colonies!

Notebook records - thoroughly describe and compare plates/treatments, target colonies

Rhizobium Media Mannitol : 10 g Yeast Extract: 1.0 g MgSO4/7H2O: 0.2 g NaCl: 0.2 g K2HPO4: 0.5 g FeCl3: 0.005 g	Prepared as agar plates (15-20 g/L). This high-sugar medium is designed to enhance capsule production, a distinctive Rhizobium trait. These plates are not particularly selective and must be used in conjunction with surface-sterilization for enrichment purposes.

LAB MATERIALS TURN-IN

11 pts. Informal Notebook records: make sure all guidelines in syllabus and above are followed.

2 pts. INDIVIDUAL TURN-IN: Each person turns in his/her final Azotobacter plate, additionally CIRCLING an Azotobacter colony. It will be graded on labeling, pattern, Azotobacter isolation/ID.

2 pts. PAIR TURN-IN: Each pair turns in their set of Rhizobium plates, circling a Rhizobium colony. It will be graded as above; make sure I can tell how each was treated!