Protease Assay K. DeAngelis October 24, 2005

Protease assays were carried out as described by Rosen (Rosen, 1957), with modifications by Ladd and Butler (1972). Because these incubations were short-term, toluene was not used to treat the soil (Ladd and Butler, 1972). Briefly, 1% casein was added to soils recovered from the rhizosphere at 0-4 cm from the root tip (root tip), 4-8 cm from the root tip (root hairs), 8-16 cm from the root tip (mature root) and soil at least 1 cm from any roots (bulk soil). Root sections were taken from the microcosms and the attached soil was extracted by rinsing in KPO_4 buffer (?) in tubes. Tubes were shaken to suspend soil and put in 100 rpm incubator shaker @ 37°C. Two time points were taken; for each, 1 mL soil solution was dispensed into 1.5 ml tubes and moved to 4°C for 30 minutes. Supernatant was recovered by centrifugation for 5 minutes at 13000 rpm. At this point the reactions are stable and can be stored at -20°C.

To estimate the rate of protease enzyme activity, the amount of casein hydrolyzed to amino acids was measured at two time points using a ninhydrin assay. Using a flat bottom 96-well plate, 40 uL of freshly prepared color reagent (one part acetate-cyanide buffer, and one part ninhydrin in DMSO) was added to 40ul of standard or sample. Plates were incubated for 30 minutes at 60°C and cooled at RT for 5 minutes before adding 160 ul 50% isopropanol and finally reading absorbance at 570 nm. Protease enzyme activity rates were calculated as conversion of added casein to glycine based on amino acid standards. Specific enzyme activity was calculated by expressing enzyme activity as a function of total bacterial cell counts per sample.

Total Bacterial Cell Counts

To normalize protease enzyme assay data as a function of bacterial cells, cells were counted using BacLight Live/Dead stain (REF).

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Actual dilution will depend on intensity of enzymes purple = aa = protease activity

dilution examples

40 uL sample / standards 8x dilution = 5 uL sample in 35 uL water
10x dilution = 4 uL sample in 36 uL water

Acetate-cyanide buffer: Sodium acetate 3.8 M pH 5.3 ($Na\text{-}C_2H_3O_2$)
= 36.0 g NaAc + $3H_2O$
+ 6.7 mL Glacial Acetic Acid
go to 100 mL with H_2O in volumetric flask

add sodium cynide (NaCN) may be stock solution in fridge
make stock: 5 mg in 10 mL H_2O (.01 M)
real time stock in fridge = 0.5 M so add 40uL per 100mL buffer

Ninhydrin Reagent: 3% weight per volume in DMSO

3 g / 100 mL (0.15 g Ninhydrine / 5 mL DMSO (Dimethyl Sulfoxide)

Ninhydrine = in dry organics zone over small centrifuge with foil over top

this forms the purple colored fun with proteins (enzymes)

also theoretically forms with ammonium, but not as fast

weight 0.15 g into small polypropylene tube

add 5 mL DMSO