Sucrose Phosphate Phosphatase (SPP) Activity Assay

Introduction

This assay tests SPP activity in our SPP-transformed *B. subtilis* cultures. The underlying principle behind the assay is that the rate of Suc-6-P to sucrose can be measured via capture of inorganic phosphate released during the conversion with an ammonium molybdate test. Phosphate ions react with ammonium molybdate to produce a colored (deep-blue) complex. The reaction is carried out in an acidic solution containing excess ascorbic acid (Vitamin C) to prevent the complex from slowly oxidising. This reaction can be applied generally for the quantitative analysis of low concentrations of PO_4^{3-} ions in solution.

Reagents

1. **Cell Buffer** (10 mL Stock)
   per 1 mL total buffer mix:
   - 0.000623g Suc6P in 1mL of buffer mix (498.46 g/mol)
   - 0.00163g MgCl2 hexahydrate in 1mL Buffer mix
   - 0.00596 g HEPES (Kosuke"s bench) in 1mL Buffer mix
2. **Reagents A & B** (** DO NOT MIX UNTIL READY FOR USE)
   - A: 100 uL of 10% ascorbic acid (store in 4°)
   - B: 600 uL of 0.42% (w/v) ammonium molybdate·4 H2O (196.01 g/mol) in 1N H2SO4 (=1/2 M H2SO4)

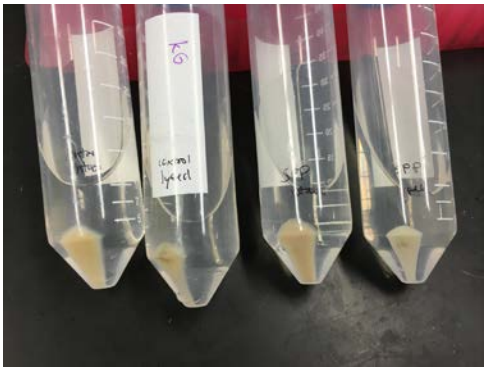
Protocol[1][2]

1. Prepare Cell Buffer, Reagents A & B.
2. Spin down (4°C, 15 min @max speed) 1) SPP-transformed Bacillus and 2) control super-competent Bacillus (same OD)
   a. 8/28: tried 10 mL of mid-saturated liquid culture of s.c. Bacillus & 10 mL of consolidated SPP cultures again; insufficient pellet size for SPP culture

   b. 8/30: We are testing 4 total samples, each grown in 25 mL. One sample for lysed cells & one for intact cells, for two sample conditions (SPP & control sc Bacillus). (can also split pellets accordingly into comparable sample sizes)

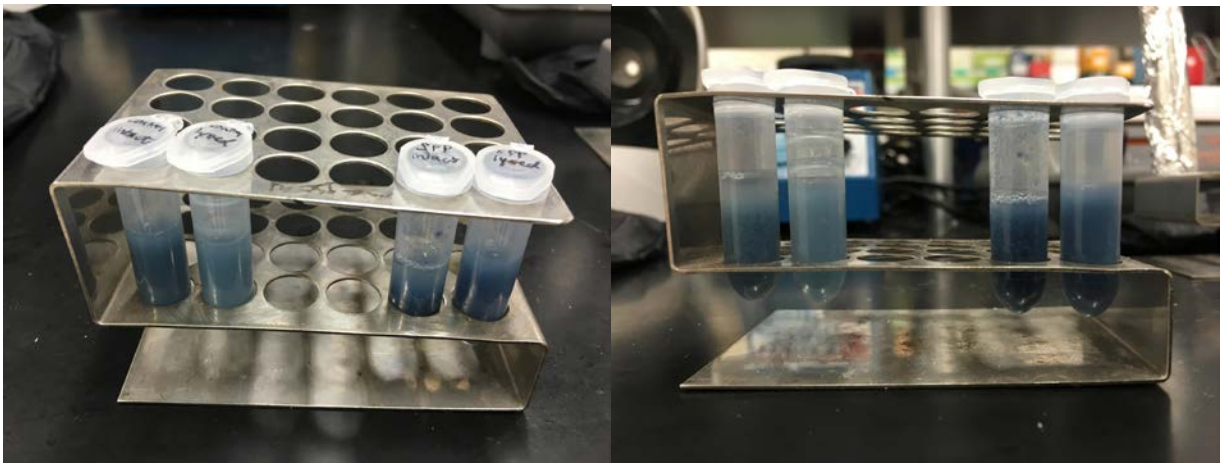
[1] Lunn J, Ashton A, Hatch M, Heldt H. "Purification, molecular cloning, and sequence analysis of sucrose-6F-phosphate phosphohydrolase from plants." *PNAS* 97(23): 12914-12919. (2000). doi: 10.1073/pnas.230430197

[2] Ames, B. "Assay of inorganic phosphate, total phosphate and phosphatases." *Methods in Enzymology* Volume 8: 115-118. (1996). doi.org/10.1016/0076-6879(66)08014-5



3. Wash pellets twice with 20 mL of saline solution (0.9% NaCl in Milli-Q) for 20 min.
4. Resuspend pooled pellets in 8 mL 0.9% saline in falcon, top off to 20 mL total of 0.9% saline
5. Lyse with sonication for 10 min over ice
    a. Sonicate every 2 min, rest for 1 min, repeat for 3 cycles
6. Spin down at max speed for 10 min and resuspend pellets in 500 uL of cell buffer solution
    a. doesn't pellet well, carefully pipette out supernatant to about 1 mL, resuspend pellet in 1 mL, transfer suspension into microcentrifuge tube

7. Incubate in cell buffer for an hour at 37°C
8. Add .7 mL of Mix A/B and incubate 20 min at 45° or 1 hour at 37°C
    a. for four tubes, make ~3 mL of Mix A/B
    b. combine: 500 uL of Mix A (10% ascorbic acid) & 3000 uL of Mix B (ammonium molybdate in H_2SO_4)
9. Vortex.
10. Read at 820 nm; 0.01 micromole of inorganic phosphate results in an absorbancy of 0.260. The color is stable for several hours. The readings are proportional to phosphate concentrations to an optical density of at least 1.8. (It is necessary that the proper phototube, sensitive to light at 820nm, be in position in the spectrophotometer; otherwise low readings will be obtained. Resulting colors are stable for several hours)



(from left to right: control intact; control lysed; SPP intact; SPP lysed. OD820 readings (blanked on control s.c. *Bacillus*): 0.790 on intact cells; 1.157 on lysed cells).

References

Ames, B. "Assay of inorganic phosphate, total phosphate and phosphatases." *Methods in Enzymology* Volume 8: 115-118. (1996). doi.org/10.1016/0076-6879(66)08014-5

Lunn J, Ashton A, Hatch M, Heldt H. "Purification, molecular cloning, and sequence analysis of sucrose-6F-phosphate phosphohydrolase from plants." PNAS 97(23): 12914-12919. (2000). doi: 10.1073/pnas.230430197