Protocol for Preparing Synthetic Nectar Solutions

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Summary:

1. Prepare a large batch of nectar solutions and store in the fridge until use.
2. Aliquot synthetic nectar into tubes, inoculate with focal yeast species, and incubate for 1 week.
3. Nectar is ready for the experiment

Materials needed:

Sucrose (99.9%) (Fisher cat # S71204)
Casamino acids (OmniPur CAS 65072-00-6)
DI water
(1) 0.2 micron filters
(1) syringe
50 mL tubes (eg. Corning, sterile)
2.0 mL tubes (sterile)
(4) 500 mL Erlenmeyer flasks
(1) 10 mL Erlenmeyer flask
(4) 0.2 micron flask filters (for use with vacuum)
plates of M. reukaufii and S. bombicola (made ~2-4 days before inoculation)

Detailed Methods:

1. Prepare amino acid stock solution (~0.5 M)
    a. Combine 1 g. of casamino acid mixture with 10 mL DI H2O in a small Erlenmeyer flask
    b. Swirl and heat gently to combine.
    c. Filter using 0.2 micron filter and syringe into a sterile tube.
    d. Store in the fridge
2. Prepare Nectar Solutions
    a. Combine the following in a large Erlenmeyer flask
    b. Heat gently in water bath to dissolve
    c. Filter using 0.2 micron flask filter into a sterile flask
    d. Aliquot 50 mL of each nectar type for each section into labeled tubes, keep in the fridge until use.

Nectar Type	Sucrose	AA Stock	Water
Low Sugar Low Amino Acid (LL)	75 g.	31.6 uL	Top up to 500 mL
Low Sugar High Amino Acid (HL)	75 g.	3.16 mL	Top up to 500 mL
High Sugar Low Amino Acid (LH)	250 g.	31.6 uL	Top up to 500 mL
High Sugar High Amino Acid (HH)	250 g.	3.16 mL	Top up to 500 mL

3. Inoculate Nectar for Experiments
    a. Prepare tubes (2 options: in green or yellow)
        i. Aliquot 1 mL of synthetic nectar into sterile 2.0 mL centrifuge tubes, with 12 tubes of each nectar type x 16 nectar types = 192 tubes/section
        ii. Alternatively, aliquot 6 mL of synthetic nectar into sterile glass culture tubes with 2 culture tubes/treatment x 16 nectar types = 32 tubes/section
    b. Prepare Yeast
        i. Pick a single colony of each yeast species and serially dilute in ~500 uL of a sterile isotonic solution (we use sterile sugar water, ~20%)
        ii. Count cells using hemacytometer and prepare 1000 cells/uL solution. (*if you need more details on this protocol, please let me know*).
    c. Inoculate
        i. Inoculate M+ and S+ tubes with 0.5 uL of M. reukaufii or S. bombicola (500 cells), respectively.
            1. 4 nectar types x 12 replicates/species= 48 tubes/species
        ii. Inoculate other tubes with 0.5 uL isotonic solution used for suspension
        iii. If using 6 mL glass culture tubes, inoculate with 3 uL yeast solution or control solution.
    d. Incubate (2 options)
        i. Incubate 1 mL tubes at 25 oC in the shaker (tape rack to bottom?)
        ii. Open tubes (carefully) to exchange O2 every couple days.
        iii. If using 6 mL glass culture tubes, leave caps loose and tape down, and shake for 7 days. We have successfully used this method many times.
    e. Nectar will be treated and ready for use!

From Tad's email:

These solutions will need to be prepared 1 week before each lab day (so, preparation on April 2 for the April 9 lab, preparation on April 3 for the two April 10 labs, etc), and all solutions (both yeast-inoculated and yeast-free solutions) should be kept at 25 C to allow yeasts to grow for 7 days.