Isolation of Ashbya from bugs

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ABSTRACT

Protocol to isolate Ashbya gossypii from milkweed and box elder bugs.

Protocol Citation: Amy Gladfelter 2023. Isolation of Ashbya from bugs. **protocols.io** https://protocols.io/view/isolation-of-ashbya-from-bugs-b56yq9fw

Protocol status: Working
We use this protocol and it's working

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1 Ashbya live in the mouths and digestive track of several species of insects, in particular the "true bugs" aka Hemiptera. Examples of some of the species native to North Carolina.

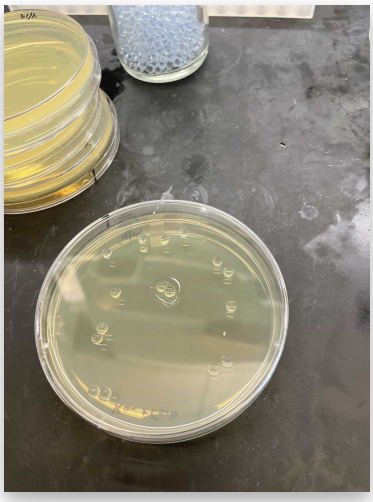
Box elder bug (adult L and larvae R). They often live in leaf litter near box elder trees (a type of maple) but also like to congregate in warm spots such as on the side of buildings.

Small milkweed bug. They live on several species of milkweed

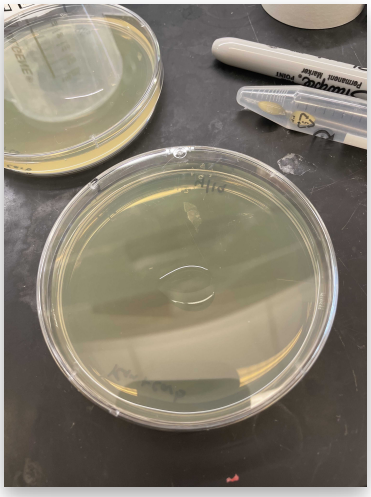
Large milkweed bug. They also live on milkweed plants

2 To collect bugs put them in any sealable container. I like to use a 50 or 15ml conical with a few small holes in the lid but I've also used clean mason jars or the disposable coffee cups at conferences. In my experience live bugs work best. If possible record the **species of insect,** the **location of collection,** the **date of collection**, and the **host plant** if there is one. If you aren't sure you can always take pictures of the bug and the plant to identify later. t

3 Prepare the plates. Use AFM with no selection. Top coat the plates with antibacterials, by adding 50ul of Amp and Kan to the top of your plate, spreading it gently and allowing it to adsorb. It doesn't have to be these specific drugs, you just want to use something to cut down the number of bacterial colonies you get.



spreading the top coat with glass beads.

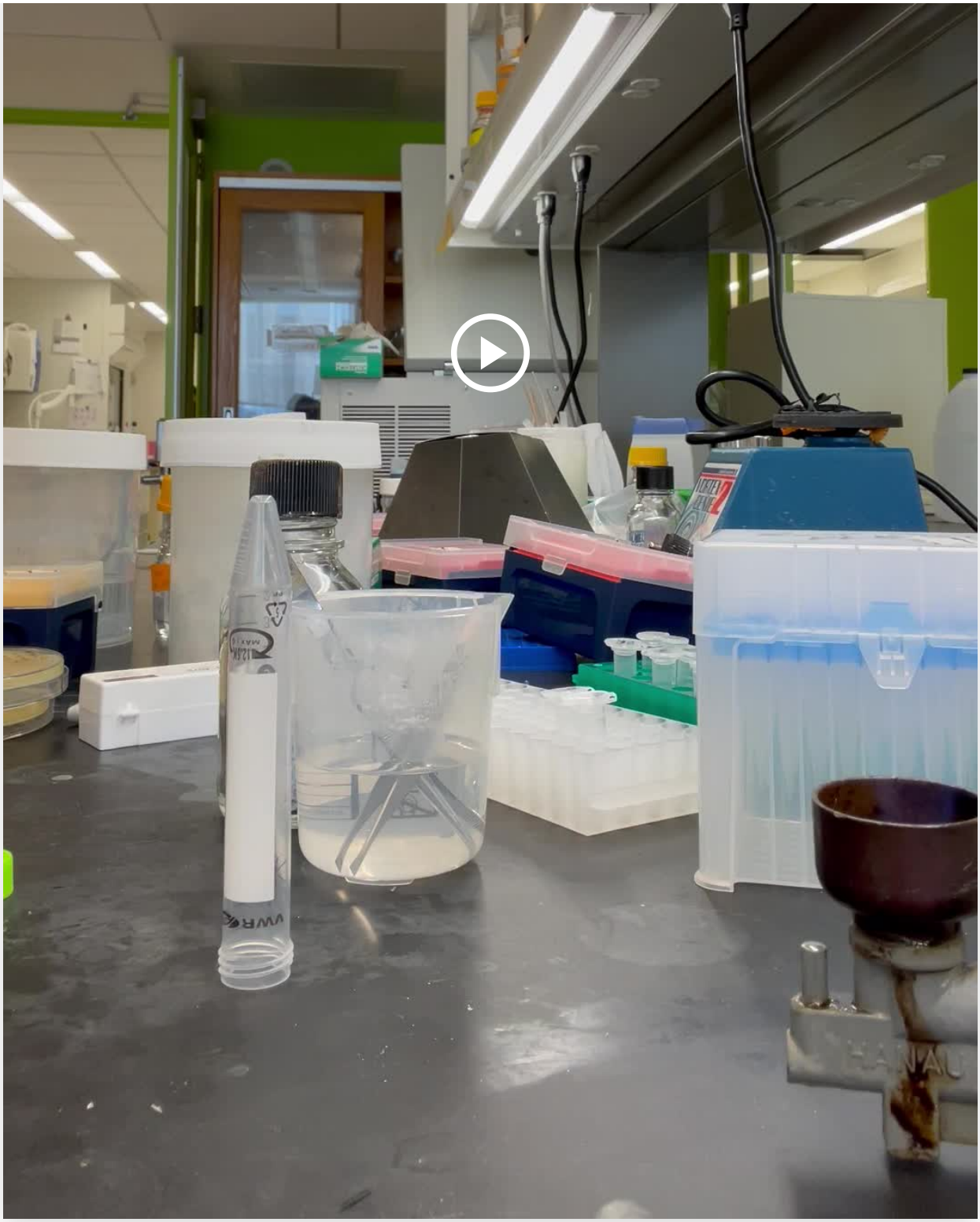


top coating with 50ul of Amp and 50ul of Kan

4 Stick the bug into the 4deg for ~30min to chill it out.

5 Quickly sterilize a pair of forceps in a flame. Remove the bug from the container. Smush the bug's head and then mash it into the agar. you can drag it around a little bit just try and make sure that it's contents end up on the agar.

6 example of the procedure.



7 Place the plate at 25 or 30deg. Check on it every day.

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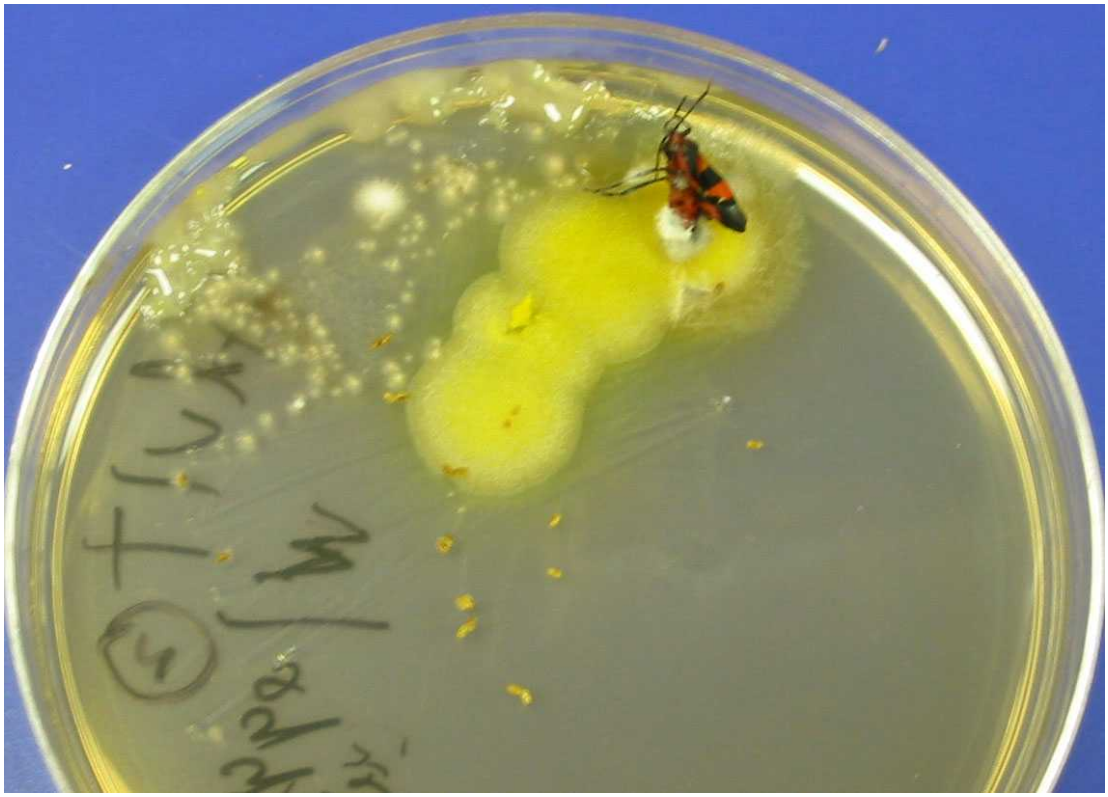

After a few days you should get a bunch of different colonies. Some are fungi and maybe some bacteria. Aim for the ones that look like ashbya, they will be flat, a little fuzzy, and might be yellow as in this example.

9

Once you get colonies pick each one to a new plate. I usually take no more than 4 from a single plate and by sequence they usually end up being the same (i.e. little to no diversity within a bug). Grow the plates until colonies are a few inches across and then make a mycelial stock and gDNA. you can determine species by sequencing with ITS primers or you can PCR the Whi3 locus and sequence that.