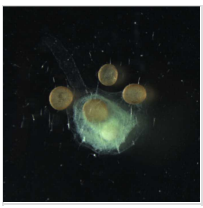

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Inducing gemmulation in the freshwater sponge *Ephydatia muelleri* in culture using theophylline

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Symbiosis Model Systems | Transfection project

April Hill

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Freshwater sponges produce overwintering cysts called gemmules that are full of stem cells, allowing them to survive harsh winter months. The gemmules of *Ephydatia muelleri* can be kept in 3°C for months, and in -80°C for years, while still remaining viable to hatch and develop into a functional sponge, as outlined in the protocol: "Hatching and freezing gemmules from the freshwater sponge *Ephydatia muelleri*" (https://dx.doi.org/10.17504/protocols.io.863hzgn). With a recently published chromosome-level assembly of the genome (Kenny *et al.*, 2020), along with multiple transcriptomes, this makes *Ephydatia muelleri* an ideal sponge species to establish as a model sponge species that can be used in labs world-wide.

While the gemmules used in the lab are usually collected from rivers and lakes every season for practical reasons, there have been studies showing the gemmulation process can be induced. A classic study looking at this process was conducted by Rasmont in 1974, who used theophylline to induce gemmulation in the freshwater sponge *Ephydatia fluviatilis*. Using up to 400uM of theophylline, this study showed that gemmulation can be induced in 100% of the sponges used in the experiments, and can occur as fast as 4 days after treatment with theophylline. While this is an interesting and important process to understand the biology of freshwater sponges, the ease and practicality of collecting masses of gemmules from wild populations most likely led to this process being rarely revisited in the lab.

Here, we outline a protocol for inducing gemmulation in the freshwater sponge *Ephydatia muelleri* using theophylline, establishedby revisiting the study done by Rasmont (1974). In *E. muelleri*, the gemmulation process takes longer than in *E. fluviatilis*, with the fastest documented gemmulation detected at 8 days after treatment with theophylline, and taking up to 12+ days in some cases. The gemmulation process usually completes within 4-5 days once the gemmulation process can be detected, and results in a smaller gemmule size than the ones obtained from wild populations. The newly formed gemmules take roughly 6-7 weeks to mature in the 3°C incubator before they can be plated to hatch.
While this is a lengthy process, being able to induce gemmulation in the lab increases the potential for using *E. muelleri* in transfection studies.

References

Kenny, N.J., Francis, W.R., Rivera-Vicéns, R.E. *et al.* Tracing animal genomic evolution with the chromosomal-level assembly of the freshwater sponge *Ephydatia muelleri*. Nature Communications. 2020. 11, 3676. https://doi.org/10.1038/s41467-020-17397-w.

Rasmont R. Stimulation of cell aggregation by theophylline in the asexual reproduction of fresh-water sponges (*Ephydatia fluviatilis*). Experientia. 1974. Jul 15;30(7):792-4. doi: 10.1007/BF01924190. PMID: 4367998.

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gemmule, gemmulation, theophylline, Ephydatia muelleri, freshwater sponge, Porifera

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Theophylline (C7H8N4O2 - MW:180.16)
Wide bore Pasteur pipettes and bulbs
Petri dishes (e.g. 5cm diameter)
Fine forceps (e.g. Dumont No.5)
10ml serological pipette

Strekal's medium:
Strekal TA, McDiffett W. 1974. Factors affecting germination, growth, and distribution of the freshwater sponge, Spongilla fragilis Leidy (Porifera). Biological Bulletin 146:267-278.

This protocol is based on an 8-gemmule sponge grown in a 5cm Petri dish (Max. volume 20ml) and treated with 400uM theophylline. Both the number of gemmules and total volume of medium can be scaled up or down.

For 10mM stock solution of theophylline:
MW:180.16
For 10 ml of 10 mM stock solution = 18.0 mg (0.018g) in 10ml of dH2O
For 50 ml of 10 mM stock solution = 90.1 mg (0.090g) in 50ml of dH2O
-> Store in 4°C (fresh is always better, but this should last for at least 1 week, up to a month at 4°C)

Hatching and growing sponges

1 Hatch and grow sponges using the protocol - "Hatching and freezing gemmules from the freshwater sponge Ephydatia muelleri" by Sally P Leys, Lauren Grombacher, and April Hill (dx.doi.org/10.17504/protocols.io.863hzgn).

1.1 Sponges grown from a larger number of gemmules have a faster rate of gemmulation and resulting number of gemmules. It is recommended to plate at least 4 gemmules close together, to grow a bigger sponge to treat with theophylline.

Treating sponges with theophylline

2 After sponges have reached stage 5 (approximately 7 days), remove culture medium to have 10mL volume in the dish. Never expose sponges to air.

2.1 **Incubate sponges in theophylline solution:**

- Make a 2x concentration of theophylline solution: add 800ul of 10mM stock solution into 10mL of 1x Strekal's medium for a final concentration of 800uM.
- Add the 10ml of 800uM to 10mL of 1x Strekal's medium containing the sponge for a final concentration of 400uM
- Cover with tin foil to protect from light.

2.2 **Change medium every 2 days:**

- Make 16 mL of 400uM theophylline solution: add 640uL of 10mM stock solution into 16mL of 1x Strekal's medium
- For each 20mL Petri dish, remove 16mL of the culture medium from the sponge dish, and replace with 16mL of the freshly made 400uM theophylline solution.

Gemmulation, maturing newly gemmulated gemmules, and removing gemmules for hatching

3 **Gemmulation**

- Under these conditions, gemmulation can be detected 1-2 weeks after the initiation of theophylline treatment.
- Early stages of gemmulation appear as white regions in the sponge, which gradually start to have a more defined shape and develop a hard casing with a light brown color in 4-5 days.

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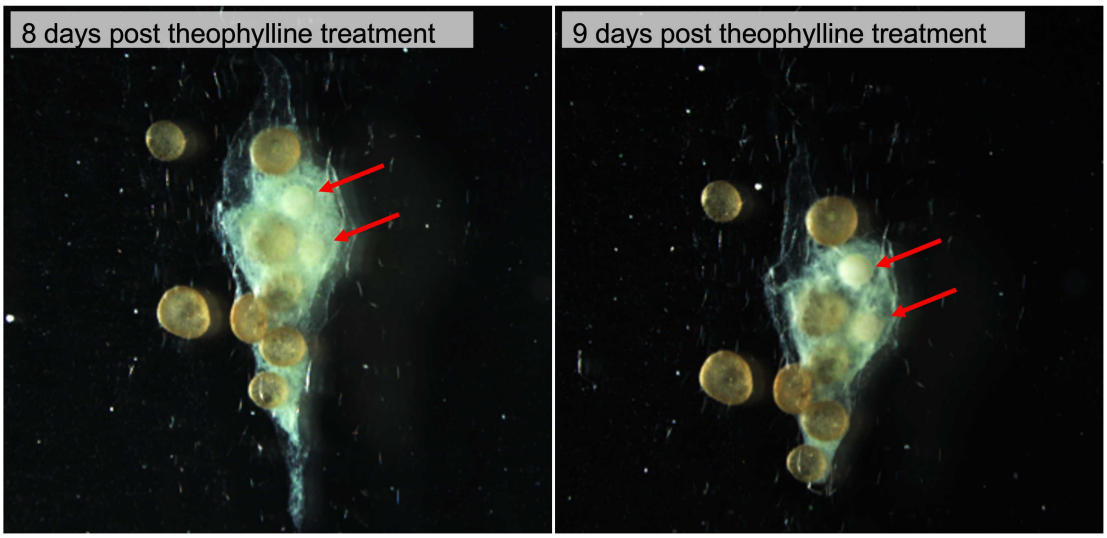

An 8 gemmule sponge at 8 and 9 days post theophylline treatment. The white 'bumps' (early sign of gemmulation) are indicated with red arrows.

3.1 Maturing newly formed gemmules

Newly formed gemmules from *Ephydatia muelleri* require maturation for 6-7 weeks before they are ready to hatch. Maturation is carried out by incubating the whole sponge at 3°C in the dark.

3.2 Removing and plating newly gemmulated gemmules

Newly formed gemmules can be removed by carefully detaching them from the sponge tissue using fine forceps (e.g. Dumont No.5), and transferring them into a new dish to hatch and grow.

At an early stage of maturation (6-7 weeks), they can take 10+ days to hatch after plating.

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