Useful Methods 2: Sterilization of Duckweed

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Sterilization of duckweed plants is unavoidable in the lab because many physiological or toxicological properties were influence in an unknown way by microorganisms, be it bacteria or fungi. Thus, the first step isolating clones from natural populations is surface sterilization. Moreover, it happens again and again that an already sterile clone gets infected during handling, even in a safety hood. Wherever axenic clones were kept under in vitro cultivation conditions, sterilization belongs to the routine techniques.

Different species (genera of species) have different sensitivity toward bleaching agents usually used for sterilization. Most sensitive are *Wolffiella* species followed by *Wolffia* species. *Lemna* is much more resistant and *Landoltia* and *Spirodela* are the most resistant.

I learned this method from the late Elias Landolt, ETH Zurich during one of my visits in his lab. Before that we kept each clone in 5 copies excluding this way that the whole clone with all copies will be infected. Now we keep usually only two copies from each clone.

For surface disinfection, we put the plants in a plastic tube (Falcon), in dependence of the size of the plants having a volume of 15 ml or 50 ml. The commercial available "*Eau de Javel*" is diluted in water for different concentrations and the fronds are treated for different periods by gentle shaking:

Wolffia, *Wolffiella*: 1 – 3 %, 2, 3, 4, and 5 min

Lemna: 3 – 5 %, 3, 4, 5, and 6 min

Landoltia, *Spirodela*: 5 – 10 %, 3, 5, 7, and 9 min.

"Eau de Javel" is available at least in Switzerland and Germany (producer Floreal Haagen, Wadgassen, Germany). It contains 2.4 % NaOCl. This means that we sterilize *Landoltia* fronds in a 0.24 % solution of NaOCl using "Eau de Javel". Thereafter, plants were transferred into Erlenmeyer flasks with nutrient medium containing sugar (50 mM glucose or 25 mM sucrose) (e.g. 180 ml per flask) without further washing in sterile water. After a few days, better after 14 days, the medium remains clear when all bacteria and fungi are killed.

Originally, we washed the plants in 70 % ethanol before bathing them in "Eau de Javel" but it turned out that this does not improve the rate of success. At the end we have typically 4 Erlenmeyer flasks with fronds treated for different times in the bleaching bath. In the cultivation room at 25oC we follow the development of the plants in these Erlenmeyer flasks.

Sterilized plants can be transferred also on Agar in Petri dishes. The Agar-layer also has to contain sugar to control the success of the sterilization. In this case, some colonies might be unsterile without infecting sterile colonies in the same Petri dish. If this method is used, washing the fronds in sterile water after sterilization is indispensable in order to remove traces of the beaching solution.

We also used other commercially available preparations like “Danklorix” (CP GABA, Hamburg, Germany), available at least in Austria and Germany. This product contains 2.8 % NaOCl and can be used in the same way as “Eau de Javel”.

Limited experience exists with commercial available sodium hypochlorit (NaOCl) solution. However, the concentration is much higher (typically 12 %) and it has to be diluted accordingly. Moreover, as it is free of surfactants, it is useful to add a small amount of a mild detergent like 0.5 % of Triton X-100 to get the surface of the whole plant body wetted.

There are other beaching agents like “Lizol” (Reckitt Benckisier, Parwanno, Himachel Pradesh, India) having e.g. 0.6 % NaOCl. However in this case, a very high concentration of surfactants is included. We were not able to wash the surfactants properly away from the surface of the plants and the recovering rates were very low.