

# Preparation of sterile medium

Making an orchid medium does not at all have to be difficult. The ingredients are simple: sugar, liquid fertilizer, potato, orange juice, agar, and demineralized water. Together these ingredients contain the necessary plant hormones, carbohydrates, amino acids, pH-buffer and inorganic nutrients.

Per litre:

Liquid fertilizer for indoor plants	3 ml
Potato (blended)	25 g
Fresh orange juice	25 ml
Sucrose (table sugar)	15 g
Household ammonia (diluted 1:10)	
Agar	7 g



Clingfilm to be sterilized in the microwave oven and medium for the pressure cooker.

Mix the ingredients and add demineralized water to a volume of 1 L. Homogenize in a kitchen blender. Measure the pH by using a cheap pH-meter from

amazon.co.uk. Adjust the pH to 5.7-5.8 by adding a few drops of dilute household ammonia at a time followed by a good stir. Choose a brand of pure household ammonia, sometimes called ammoniac, without added detergent. Dilute the household ammonia 1:10 with water before use. If the pH becomes too high, lowered again by adding a few ml of orange juice. Then boil to dissolve the agar.



Pressure cooker for sterilization of the medium.

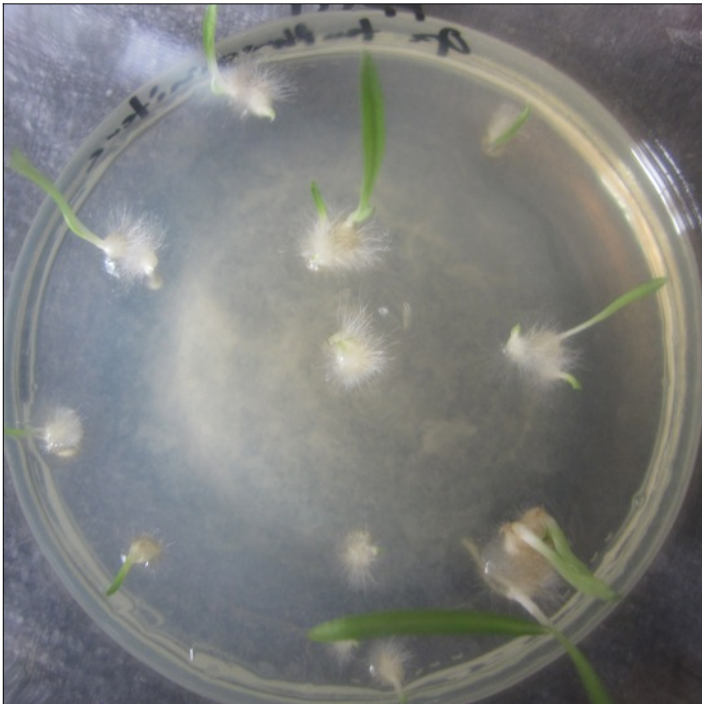
Pour the hot medium into the flasks, about 2 cm, cap with two layers of aluminium foil and boil in a kitchen pressure cooker for 30 min at maximum pressure with sufficient water in the cooker. Store the flasks in closed plastic bags until use. Tomato sauce jars or jam jars are good culture flasks especially if they have narrow tops that reduce the risk of contamination. Remove all traces of glue (from the labels) before using the flasks. This is because toxic compounds from the glue may be released during sterilization and enter the medium.

The liquid mineral fertilizer should have a total nitrogen content of 5% to 6% (containing both ammonium-N and nitrate-N) and should also contain micro-nutrients. I use a Danish brand called Substral with the following content: nitrogen 6% (nitrate-N 3.3%, ammonium-N 2.7%), phosphorous 1.3%, potassium 5%, boron 0.01%, copper 0.005%, iron 0.03%, manganese 0.01% zinc 0.002%, trace elements in the fertilizer are chelated with EDTA and DTPA to keep them in solution.

Agar can be bought from amazon.co.uk, I use the brand SpecialIngredients. Wash the agar in water before use to remove chloride and other compounds that are toxic for the small orchids. Enzymes (proteases) in the fresh orange juice will hydrolyze some of the protein in the blended potato, this will provide peptides and free amino acids for the germinating seeds.



Wash the agar by suspending the agar flakes in tap water. Let the flakes sink to the bottom for 5 min and decant the water.



*Ophrys tenthredinifera*, first year on liquid fertilizer medium.

I started out with a more complex recipe, but have gradually made it more simple as there seems to be little or no effect of compounds such as myo-inositol, adenine, peptone, and pure lab-grade mineral nutrients vs. a general liquid fertiliser for potted plants

Almost all pictures on my homepage are of plants germinated on the fertilizer medium, but some species have been reported to be very sensitive to inorganic nitrogen (1). If there are indications of toxicity, I also sow on a medium with inorganic nitrogen in the form of the amino acid glycine, a medium inspired by Svante Malmgren (2). Germination and growth, however, seems to be slower on the glycine medium for most species. My glycine medium has the following composition:

Per litre:

CaCl <sub>2</sub> - 2H <sub>2</sub> O	50 mg
K <sub>2</sub> HPO <sub>4</sub>	100 mg
MgSO <sub>4</sub> - 7H <sub>2</sub> O	50 mg
FeSO <sub>4</sub> - 7H <sub>2</sub> O	20 mg
MnSO <sub>4</sub> - 1H <sub>2</sub> O	10 mg
Glycine	700 mg
Potato (blended)	25 g
Fresh orange juice	25 ml
Sucrose (table sugar)	15 g
Agar	7 g

The medium should be prepared as described above for the liquid fertilizer medium. Pure glycine is easy to obtain, I use a food-grade product made by a company called Bulk Powders. CaCl<sub>2</sub> (calcium chloride), K<sub>2</sub>HPO<sub>4</sub> (potassium phosphate, dibasic, dipotassium phosphate), MgSO<sub>4</sub> (magnesium sulphate), MnSO<sub>4</sub> (manganese sulphate) and glycine can be obtained from [www.amazon.co.uk](http://www.amazon.co.uk). It can be convenient to make one liter of a 10x concentrated stock solution so that you do not need a laboratory scale for weighing. Store the stock solution in 100-mL aliquots in the freezer and mix with 900 mL demineralized water right before autoclaving the flasks in the pressure cooker .

#### References:

1. Ponert J et al. 2013 Asymbiotic germination of mature seeds and protocorm development of *Pseudorchis albida* (*Orchidaceae*) are inhibited by nitrates even at extremely low concentrations. *Botany* 91: 662-670.
2. Malmgren, S. 1993. Asymbiotisk fröförökning i stor skala av *Anacamptis*, *Ophrys*, *Orchis* och andra orkideer med runda rodknölar. *Svensk Botanisk Tidskrift* 87: 221-234.



*Satyrium nepalense*, second year on liquid fertilizer medium.



Substral liquid fertilizer for indoor plants. A Danish brand that may be difficult to find abroad but any high-quality liquid mineral fertilizer with 5% to 6% total N and micro-nutrients should be OK.



Agar-quality is important. Agar from Asian food shops is sometimes low-quality and toxic for the protocorms. I use the agar brand SpecialIngredients bought from Amazon or at <https://www.specialingredients.co.uk/>



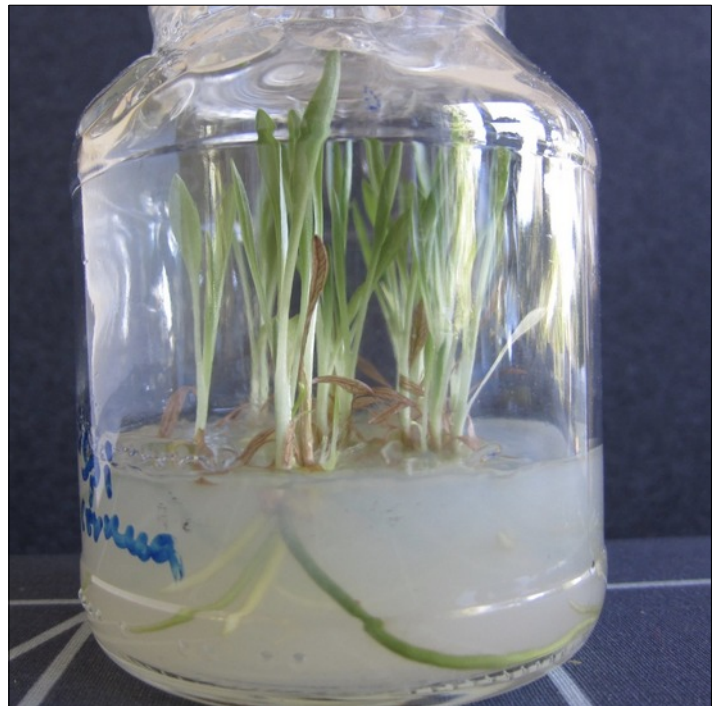
The liquid fertilizer medium also works well for indoor epiphytes like these *Phalaenopsis* seedlings.



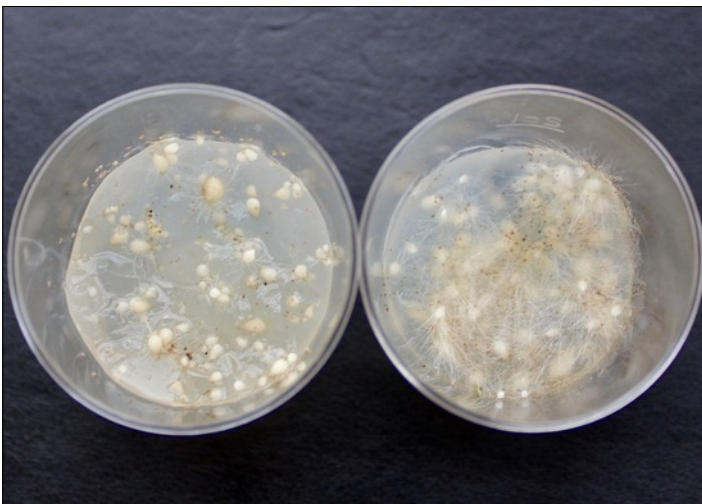
A cheap but very precise scale (Palmscale 8.0, 55 €). Other models can be found at [amazon.co.uk](http://amazon.co.uk). The precision should be 0.01 g.



*Thelymitra rubra* and *Thelymitra pauciflora* seedlings ready for reflasking.



*Spiranthes cernua* ready for deflasking



*Dactylorhiza maculata* and *Orchis anatolica* protocorms ready for reflasking.