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Protocol of lettuce juice culture medium for *Paramecium*

National BioResource Project (NBRP) *Paramecium*　Yamaguchi Univ. Masahiro FUJISHIMA

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1. Measure weight of the lettuce leaves[*1](see attached photos of the usable lettuces).
2. Wash the leaves by tap water by hand, then rinse with deionized water twice.
3. Boil the leaves for 1-2 min to inactivate various enzymes of the leaves, then cool with ice-cold deionized water.
4. Homogenize the leaves with a home juicer.
5. Filtrate the homogenates with 8 layers of gauze by squeezing.
6. Dilute the lettuce juice with deionized water. If the weight of the lettuce leaves of above 1 was 1 kg, make the juice two litters by adding deionized water.
7. Store the juice in freezer until use, or perform intermittent sterilization at 105 ℃ for 5 minutes once a day, place at room temperature overnight and repeat for 3 days. The lettuce juice stock solution can be kept at 4℃ or room temperature. Also, this stock solution can be kept at -20℃ or -30 ℃ without intermittent sterilization. At the time of use, thaw, filter through two sheets of kimwipes, and the filtrate is used.
8. Prepare the stock solution of Ca-free modified Dryl's solution, in which Na_2HPO_4 $12H_2O$ is replaced with KH_2PO_4,, and store at 4℃ until use.

$C_6H_5Na_30_7 \cdot 2H_2O$	88.2 g
$NaH_2PO_4 \cdot 2H_2O$	75.2 g
KH_2PO_4	12.3 g
Deionized water	add to 3 L

9. Prepare stock solution of 200 mM $CaCl_2$, dispense 12 mL each in 18 x 180 mm glass tubes, cover with aluminum cap, autoclave, and keep at room temperature until use.
10. Mix 40 mL lettuce juice stock solution, 32 mL stock solution of Ca-free modified Dryl's solution, 1516 mL of deisonized water in 2 L flask, cover with a silicon plug and aluminum foil, autoclave, and keep at room temperature until use..
11. Add stock solution of $CaCl_2$ to the solution of 9 in a clean bench and inoculate food bacteria of *Klebsiella pneumoniae* (BSL 2) strain 6081 or *Enterobacter aerogenes* (BSL 1) ATCC35028 one day before use. These bacteria grow well on agar for general bacteria at 24-25℃.

Final concentrations

Lettuce juice	1.25% (w/v)
$C_6H_5Na_3O_7 \cdot 2H_2O$	2.0 mM
$NaH_2PO_4 \cdot 12H_2O$	1.4 mM
KH_2PO_4	0.6 mM
$CaCl_2$	1.5 mM

For ordinary cultures with 18 x 180 mm tubes, few hundreds micro liters of culture medium containing several hundred paramecia are inoculated into 2 mL fresh culture medium; then 4, 4, 6, 6, and 6 ml of fresh culture medium are added successively over 5 days in case of *Paramecium bursaria*. Cultures are maintained at 24 - 25℃ under a fluorescent light (about 1,500 lux). For fast grow species including *P. caudatum*, 2, 4, 10, 10 ml of fresh culture medium are added successively, and light is not needed. One or two days after the final feeding, the cultures are in the early stationary phase of growth, and express strong mating reactivity.

For mass cultures, 2 L, 5 L flusks or 10 L polystyrene latex tanks are convenient. One day after inoculation of food bacteria to these flusk or tank, few tube cultures of stationary phase of growth are added and shake the culture vessels gently by hands for a few seconds in the morning and evening to disperse the cells uniformly.

See convenient centrifuge tubes and nilon-mesh filters for concentration and for washing of paramecia.*2

*1 **Lettuce usable for *Paramecium* culture medium.**

Fig. 1 Boston lettuce. This is good for paramecia. Original lettuce juice culture medium uses this lettuce (Hiwatashi , *Gnetics* 58, 373-386, 1968).

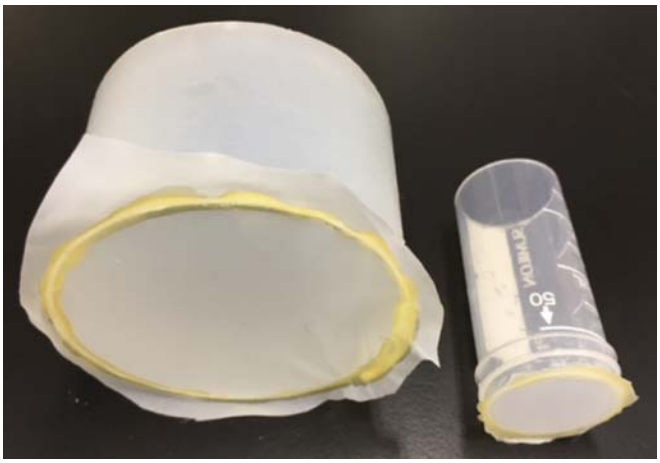
Fig. 2 Green leaf lettuce, *Lactuca sativa*. This is also good, cheep, and used first in Fujishima et al. *Zoological Science* 7, 849–860, 1990.

Fig. 3 Red-tip leaf lettuce (*Lactuca sativa var. crispa*). Paramecia do not like this one.

*2 **Convenient glass centrifuge tubes and hand-made nilon-mesh filters for concentration and for washing of paramecia.**

Centrifuge tube: From left, a small centrifuge tube made with a Pasteur pipette. A 10 mL galass centrifuge tube with an about 0.4 mL protruding tip. A 100 mL glass centrifuge with an about 2 mL protruding tip. The two centrifuge tubes from the left are used by hand-operated centrifuge, and third one is used by oil separation centrifuge . Paramecia are trapped to the centrifuge tubes after centrufugation.

Nylon mesh filter : 15 μm pore size filter was attached to a plastic tube with a water proof adhesive.



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