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A new method for pure culture of macroscopic algae, the one step selection method*

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The axenic culture is useful not only for studies of algal nutrition but also for providing materials for clarifying many aspects of algal morphogenesis. The purification methods of macroscopic algae were summarized by CHAPMAN (1973) and TATEWAKI (1979). The pipette washing method, a basic one, is usually used for isolation of axenic strains, but skilled technique is required for this method. And it takes much trouble to obtain large amount of axenic strains. On the other hand, some methods employing chemical sterilants for obtaining an axenic culture were published: antibiotics (PROVASOLI 1958, BOALCH 1961, IWASAKI 1961, TATEWAKI and PROVASOLI 1964); iodine (FRIES 1963); potassium tellurite (DUCKER and WILLOGHBY 1964); sulfa drugs (KANAZAWA 1968); and antibiotics and sodium hypochlorite in combination (DRUEHL and HSIAO 1969). These methods were convenient, but were insufficient in regard to the efficiency of sterilization and toxicity of chemicals.

Following is a description of a new method for obtaining axenic cultures more conveniently and securely. Using the one step selection method, axenic algal strains will be able to be selected simultaneously with a sterility test.

The alga tested was *Dictyosiphon foeniculaceus* (Dictyosiphonales, Phaeophyta) from the beach of Charatsunai, Muroran, Hokkaido. Plants were collected during June of 1977. The antibiotic mixture (ABM) described by GUILLARD (1968) was employed. The composition of ABM was 124.5 mg penicillin G, 50 mg streptomycine sulphate and 20 mg chloramphenicol dissolved in 1 liter autoclaved sea water. The ABM solution was sterilized by passing through Millipore filters of 0.22 μm pore size. These antibiotics were manufactured by Sigma Chemical Co., St. Louis, Missori, U.S.A. The culture medium used was ASP 12-NTA (PROVASOLI 1963). Agar plates used for the selection of axenic strains were 90×20 mm petri dishes containing 20 m*l* ST3 medium (PROVASOLI *et al.* 1957) solidified with 1.0-1.5% agar. The sterility test media used were ST3, ASP-Bl and bouillon media. The composition of ASP-Bl medium was 1 liter ASP 12-NTA, 5 g bactopepton, 2g beef extract, 0.5 g KNO_3 and pH 7.8-8.0, and the composition of bouillon medium was 0.7 liter sea water, 0.3 liter pure water, 20 g powdered bouillon and pH 7.5-8.0. Bactopepton and beef extract were manufactured by Difco Labolatories, Detroit, Michigan, U.S.A. Powdered bouillon was manufactured by Kyokuto Seiyaku Kogyo Co., Tokyo, Japan. The ASP 12-NTA and agar plate

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Table 1. Sterility tests using ST3, ASP-Bl and bouillon media.

Number of tested strains	Number of contaminated strains			Number of axenic strains	Axenic ratio
	ST3	ASP-Bl	Bouillon		
40	0	2*	2*	38	95%

A few pieces of every one of the forty strains were inoculated in every test medium.
* These were the results of two strains (nos. DF-015, 017), and each strain is recognized as contaminative in the two media.

cultures were incubated at 14°C under an illumination by cool white fluorescent lamps at 2,000 lx with 14 : 10 hr light-dark cycle. The sterility test cultures were carried out in a dark at 20°C.

The axenic culture was established by following procedures:

1. Mature materials were wiped with a clean gauze, rinsed several times in autoclaved sea water and cut into a length of ca. 1 cm.

2. Ten pieces of materials were transferred into a glass vessel containing 100 m*l* ABM and stocked in a refrigerator regulated 5°C for 2 days.

3. One piece was rinsed several times in sterile ASP 12-NTA medium, and placed in 60×15 mm petri dish containing 10 m*l* sterile ASP 12-NTA medium. Numerous zoospores were released within 10 minutes.

4. Two or three drops of the medium containing many zoospores were put on agar plate with a pipette, and spread over an agar surface with a glass stick. The agar plate was cultured for 1 month.

5. After 1 month the germlings developed into 0.5-1.0 mm masses of profusely branched filamentous thalli, and bacteria which survived in no. 2 treatment grew to 3-5 mm diameter colonies (Fig. 1). Clean algal masses growing on an agar plate were selected and inoculated into 10 m*l* ASP 12-NTA medium in test tubes. This treatment served both an isolation of axenic algal strains and a sterility test of bacteria.

6. These cultures were maintained in an incubator for 1 month, and ca. 3 mm masses of axenic microthalli were obtained (Figs. 2, 3).

The procedure is shown by the following diagram (Fig. 4).

Moreover, sterility tests were tried using ST3, ASP-Bl and bouillon media. The cultures were considered to be axenic if the test media remained clear, and contaminative if the test media became cloudy for

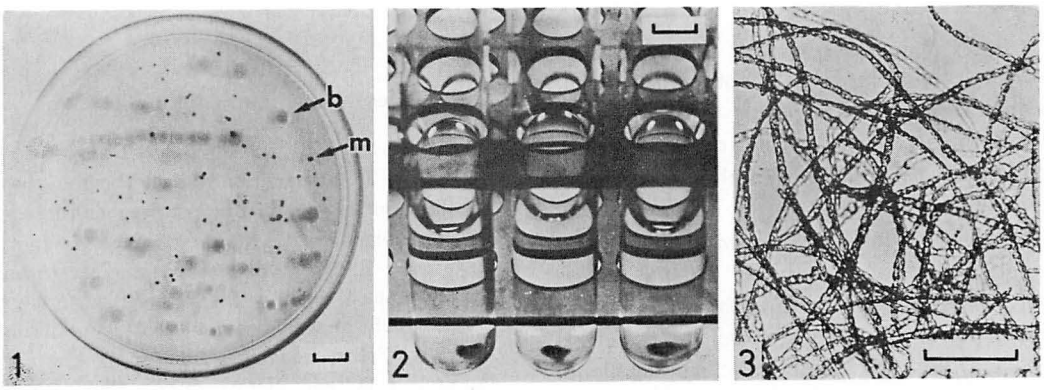

Fig. 1. Agar plate maintained for 1 month (b=colony of bactria; m=mass of microthallus); Figs. 2-3. Axenic microthalli cultured in ASP 12-NTA for 1 month. Scale 1 and 2 show 1 cm; scale 3 shows 100 μm.

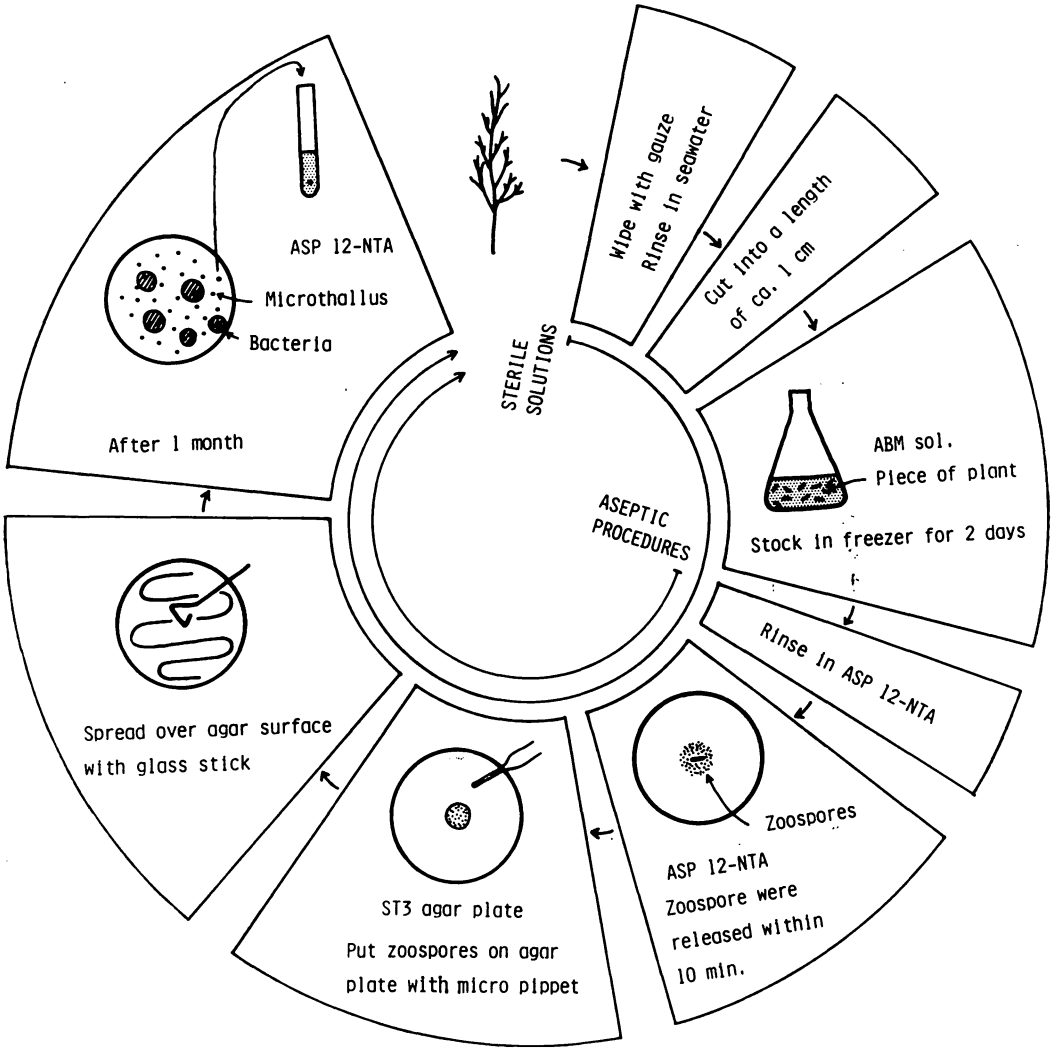

Fig. 4. Diagram summarizing procedures of one step selection method.

2-3 months. The results obtained by this method are given in table 1. This series of treatments was carried out repeatedly, and axenic strains were always obtained more than 90%.

Axenic strains of *Laminaria angustata*, *Pogotrichum yezoense* and *Ulva pertusa* were also be obtained in high ratio by using this method. The one step selection method reported here is useful and applicable to other macroscopic or microscopic algae.

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嵯峨直恒・阪井與志雄：大形藻類の無菌培養のための新しい方法，一段階選択法について

細菌検索用培地を利用した新しい方法により，ウイキョウモの無菌株と混菌株を一段階で肉眼により選別できた。この一段階選択法により，ミツイシコンブ，コブノヒゲ，アナアオサでも無菌株を高率で得ることができた。この方法は他の大形あるいは小形の藻類にも適用できると思われる。 (051 北海道室蘭市母恋南町1-13 北海道大学理学部附属海藻研究施設)