ISOLATION OF LIVING ALGAE GROWING IN THE SHELLS OF MOLLUSCS AND BARNACLES WITH E D T A (ETHYLENEDIAMINETETRAACETIC ACID)

W. F. PRUD'HOMME VAN REINE and C. VAN DEN HOEK

INTRODUCTION

Several decalcifying mixtures or aqueous solutions of inorganic or organic acids are generally used for releasing algae growing in the shells of molluscs and barnacles, for instance dilute hydrochloric, nitric, citric, or acetic acid (4), a mixture of nitric acid, chromic acid and alcolhol (1), nitric acid and alcohol (9), chlorine dioxide and acetic acid (diaphanol) (3, 6, 11, 12), and formic acid and formiate (8). For a review see (7). Such mixtures or solutions generate carbondioxide bubbles that more or less disorganise the histological structure of the thallus. They also hydrolyse cell-contents and cell-wall material. Diaphanol and formiate appeared to be relatively useful for conserving the histological structure of the thalli. Recently (5, 10, 13) chelating agents, e.g. EDTA (ethylenediaminetetraacetic acid) were introduced as histological decalcifiers. The fact that EDTA is widely used as a chelating ingredient of nutrient media for algal cultures suggested its possible use for releasing living algae growing in animal shells. However, the concentration necessary for decalcification is a multiple of that used in culture media (1 % to 5 % and 0.0005 %, respectively). It is possible to vary the pH from 5—10, in accordance with the pH of the natural habitat of the alga.

METHOD

Three perforating algal species, *Eugomontia sacculata*, *Gomontia polyrhiza*, and *Porphyra purpurea (Conchocelis*-phase), were unialgally cultured in fragments of oyster- or egg-shells placed in culture tubes with Erdschreiber-medium (2). Diminutive algae-infected fragments (*c.* 2 × 2 mm) were decalcified in 5 %, 2.5 %, and 1 % Na_2-EDTA (Fluka) solutions in Erdschreiber contained in 5 cc glass-tubes. The pH of these solutions was adjusted to 7.0 with NaOH and HCl. The tubes were exposed to 18 hours daily photo-periods in culture chambers of 4°, 12°, and 20° C. (The temperature-differences did not have any noticeable effect on survival.) From the never completely decalcified fragments surviving algae were isolated and transferred to fresh Erdschreiber.

RESULTS AND DISCUSSION

It is indeed possible to obtain living algae growing in animal shells by this method. *Conchocelis* is apparently more sensitive to EDTA than both *Chlorophyceae*. Some experiments with *Cyanophyceae*-infected shells *(Entophysalis deusta, Plectonema spp.)* indicate a still much higher resistance of these algae against EDTA; after three weeks in 5 % EDTA-Erdschreiber they were still living.

% EDTA in Erdschreiber	5 %	2½ %	1 %
Eugomontia sacculata	1	1	>14
Gomontia polyrhiza	2	>14	>14
Conchocelis	<1	<1	3

Table 1. Indicated is for each species the number of days it survives at least in the corresponding concentrations.

Very high EDTA concentrations in seawater are needed to obtain only a partial decalcification of very small fragments (a 5 % solution is almost saturated), very probably as a result of the complex-former being occupied by ions from the medium. Fragments of comparable size are readily and completely decalcified in 5 % EDTA-solution in aqua dest. within 24 hours. Possibly the EDTA-solution can be more effectively used by suspending the shell-fragments in the toplayer; the relatively heavy Ca-EDTA-complex will sink to the bottom and cause a circulation (5). These high concentrations probably bind many substances that are important for the algae, which, at best, survive this disagreeable treatment.

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