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GROWTH AND DIFFERENTIATION OF *ULVA RIGIDA* C. AGARDH *IN VITRO*

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The *in vitro* growth and differentiation of the alga *Ulva rigida* C. Agardh have been studied.

Plants grown in enriched sea water (medium II and especially medium L) have a higher chlorophyll content and regularly release a large number of reproductive cells (in 10 days). This capability does not depend on the collecting season.

It has been confirmed that axenic *Ulva* cultures grown in solution degenerate. However, if the isolated germlings were grown on semi-solid media their differentiation sometimes took place. Attachment of the germlings to the fragment of the mother plant seems also to promote differentiation.

It was found that *Ulva rigida* could better tolerate a lower salinity than a higher one.

Introduction

The interest in study of multicellular marine algae has recently been increasing. Several sea weeds such as *Ulva* (sea lettuce) are suitable for food and feed; they are also used as fertilizers and for different medical and industrial purposes (see Starr 1971, Hoppe et al., 1979). Because of its high protein content (26% of protein on air dried basis; Sitakararao and Tipnis 1964) *Ulva* could also serve as a potential source of protein.

Culture and sporulating ability of different *Ulva* species have been investigated by several authors (e.g. Provasoli 1063, Løvlie 1969, Subbaramaiah 1970, Nordby and Hoxmark 1972, Nilsen and Nordby 1975). Comparatively little work was done on *Ulva rigida*, earlier *Ulva lactuca* var. *rigida* the most common species on the Mediter-

ranean coast (see survey on *Ulvales* by Bliding 1968). The effect of IAA on the growth of the same alga was studied by Kale and Krishnamurthy 1969).

The purpose of this study was to investigate the *in vitro* growth and differentiation of this alga.

Material and Methods

Actively growing *Ulva rigida* C. Agardh* plants were collected in the coastal area from the shore of Crikvenica and Biograd n/m in November 1979, as well as in May and September 1980.

Two kinds of starting material were used: a) discoidal thallus fragments of differentiated *Ulva* and b) germlings produced *in vitro*.

The algal material was sterilized by UV irradiation (9 min., distance 52 cm, »Sterisol« UV Hanau F1137 4H lamp) and cultivated under white fluorescent light (2200 or 2700 lx, with a photoperiod of 14 hours at 20 or 26° C. »Hazocid« (0.3 and 0.5 per cent) and mixture of antibiotics (penicillin + streptomycin) proved insufficient.

Culture media used:

1. Filtered sterilized sea water
2. Medium II (Strand et al. 1916)
3. Medium L (Løvlie 1969).

Chlorophylls a and b were extracted according to Arnon (Arnon 1949, cit. Holden 1965) from *Ulva* fragments previously blotted between sheets of tissue paper.

Results

Thallus fragments

A comparison between the thallus fragments cultivated in different media was made, with respect to chlorophyll content, and the possibility of germling induction.

After 30 days of cultivation in natural sea water the deep green fragments became pale green. As shown in Fig. 1 both chlorophyll a and b contents decreased significantly (0.7 and 0.5 mg per fragment respectively). Plants grown in this medium released germlings only after 50 — 100 days.

However, in the other solutions (medium II and L) lettuce remained deep green during the same period of time. The content of chlorophyll a and b increased in time (especially in the medium L, Fig. 1). After approximately 10 days the plants grown in solutions II and L regularly released large numbers of reproductive cells. Motile two-flagellated swarmers (presumably gametes) were observed only two times. In most cases already young filamentous germlings, which were attached to

* We wish to thank Dr. A. Span for determination of the species.

the bottom of the Petri dishes, were observed. Vegetative cells from the border of the disc after division often germinated directly without loosing contact to the mother plant. In that case the disc became surrounded by round solid filaments which in some cases differentiated into blade-like thallus typical for *Ulva*. This alga's capability to form germlings did not depend on the collecting season or on the time of the year when the experiments were carried out. Every time the alga was cultivated in medium II or L the germlings appeared within 10 days. The same effect could be obtained when the fragments, which did not produce the swarmers in the natural sea water, were transfered to medium II or L.

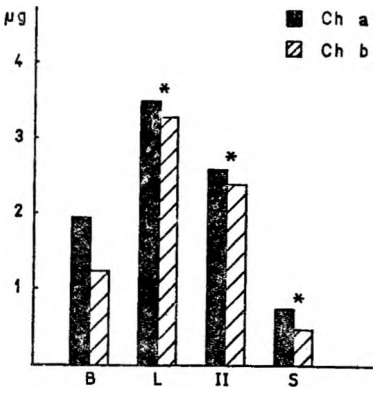

Fig. 1. Amount of chlorophyll a and b per discoidal thallus fragment of Ulva. B = at the beginning of the experiment; L = in medium L; II = in medium II; S = in natural sea water.

* Statistically significant p 0.01 according to Student's t test. Columns represent a mean of 10 measurements.

Growth of Ulva germlings

The germlings obtained in our experiments always appeared simultaneously and were therefore of the same age and size. This made them an excellent starting material for the investigations of the optimal conditions for *Ulva* growth and differentiation.

Concerning the temperature, *Ulva* germlings grew faster at 26° C when compared to lower temperatures (20° C). However, since the germlings quickly degenerate at the higher temperatures only lower temperatures were used for further experiments.

Effect of IAA on the growth of germlings

Germlings of the same size were, after washing in sterilized water, cultivated in various culture media: medium II, and medium II with indol acetic acid (IAA). The 50 µg l^{-1} concentration of IAA was found optimal according to Provasoli and Carlucci (1974). In the two solutions the germlings were of the same shape but of different length. Their average length in medium II was about 750 µm and 820 µm in IAA enriched medium II.

Effect of salinity on the growth of germlings

For this purpose the germlings were cultivated in natural sea water, diluted sea water (24‰, 50‰ and 76‰) and concentrated sea water (with 35 or 70 mg/ml NaCl added). The highest percent of survival was found in hyposaline conditions. However, there was no differentiation of the thallus either in this, or in the other solutions. In hyposaline water all the germlings were attached to the bottom of the plate, while in the hypersaline water not one single germling was attached.

An interesting feature of every germling is a colourless rhizoid, which later on multiplies and ramifies. Its purpose is to attach the germling to the base. When these rhizoids were cut and cultivated in medium II and illuminated, they become green and started to grow. In some cases they showed even some differentiation into several green tubular filaments and ramified rhizoids. This effect was particularly pronounced when the rhizoids were cultivated on a semi-solid medium. Drops of different sizes could usually be seen on their surface, even macroscopically. When the drops were collected and dried a great number of crystals appeared, which most probably were pure NaCl secreted from the cells.

Despite the improved growth conditions and intense growth in our experiments the differentiation of germlings in a liquid medium (medium II) never occured. On the other hand, the differentiation of small plants on a semi-solid medium (medium II + 1‰ agar) sometimes took place, although it was also rather rare.

Discussion

The material used in this study corresponds to the description of *Ulva rigida* C. Agardh (Bliding 1968) — the thallus had toothlike, generally microscopic protuberances, and the cells in surface view were rectangular or slightly polygonally rounded with the average measures 18 × 14 μm for a fully grown cell.

With respect to the chlorophyll content and the general aspect of the alga fragments cultivated in different media, it could be concluded that natural sea water is not enough for the alga cultivation. Plants grown in enriched sea water (medium II and especially medium L), not only have a higher chlorophyll content, but also regularly induce the release of large number of reproductive cells (after approximately 10 days). According to Subbaramaiah (1970) the *Ulva fasciata* germlings generate in nature and in laboratory conditions only during the summer low tide. Our results indicate that this alga's capability to form germlings does not depend on the collecting season or the time of the year when the experiments are carried out. It depends mostly on the composition of the medium.

According to Mohsen et al. (1974) the best range of salinity concentrations stimulating the increase in both dry weight and total nitrogen lies between 20 and 35‰. Lower salinities, on the other hand, below 20‰ retard both the maturation and discharge of all kinds of swarmers. Also under higher salinities (40 — 45‰) the production of zooids seems to be completely eliminated. The increase in salinity results in the increase in sugar content, i. e. in the increase of the rate of photosynthesis (Legendre 1921, Matsui and Matsui 1965). This work confirms the fact that this lettuce is eurihaline. It was found out that the lower sali-

nity (diluted sea water) could be tolerated better then the higher one. Most tolerant for the high salinity seem to be the rhizoids. The observed secretion of salt on their surface could be a way of osmoregulation.

A few workers have shown that axenic *Ulva* cultures, when the natural accompaniyng microflora was removed, almost always degenerate (see Provasoli and Carlucci 1974). The typical thallus has very rarely been found and our results prove the same. That has been attributed to the lack of morphogenetic regulators which are presumably produced by microorganisms (Provasoli and Pinter 1964, Waite and Mitchell 1976, Harrison 1978).

Although we have found fairly good growth conditions for the germlings, they never differentiated in the liquid medium, not even with the addition of IAA. So, the differentiation which sometimes took place on a semisolid medium could perhaps be ascribed to the germlings position on the agar surface where they were in constant contact with air. This finding could be connected with the low tide which stimulates the germlings to differentiate. Attachment of germlings to disc-fragments of alga also promoted differentiation of the thallus. It is possible that the alga itself produces some auxin-like substances necessary for normal growth and differentiation.

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SAŽETAK

RASTENJE I DIFERENCIJACIJA ALGE *ULVA RIGIDA* C. AGARDH *IN VITRO*

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Istraženi su rastenje i mogućnost diferencijacije alge *Ulva rigida* C. Agardh u *in vitro* uvjetima.

Najpovoljniji medij za rastenje alge je solima obogaćena morska voda (medij II i naročito medij L). Osim što *Ulva* u tim otopinama sadržava povećanu količinu klorofila, ona redovito nakon 10 dana oslobađa velik broj mladih klica. Stvaranje klica ne ovisi o godišnjem dobu, već isključivo o uzgojnoj podlozi.

Potvrđeno je da u posve akseničnim uvjetima alga degenerira. Ipak, klice se ponekad diferenciraju u normalan talus, ako se uzgajaju na polukrutoj podlozi. Povezanost klica s talusom odrasle alge povoljno djeluje na rastenje i diferencijaciju.

Ulva rigida je eurihalina, a bolje podnosi niži salinitet od višeg. Primijećeno je da rizoidi uzgojeni u kulturi izlučuju NaCl te time vjerojatno vrše osmoregulaciju stanica.

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