Agata BARTKIEWICZ, Tadeusz WILCZOK

Oceanologia, 17 (1983)
PL ISSN 0078-3234

Silesian Medical Academy
Institute of Chemistry and Physics — Sosnowiec

CULTIVATION OF ACETABULARIA CALYCULUS ON COMPLETELY ARTIFICIAL MEDIUM

Contents: 1. Introduction, 2. Material and methods, 3. Results, 4. Discussion; Streszczenie; References

1. INTRODUCTION

It is known that the South Japan Sea is the natural environment of the unicellular alga Acetabularia calyculus. For cultivation under artificial conditions the Erdschreiberlösung (ESL) with natural sea-water as described by Hämmerling [8] has been used in principle, for the Acetabularia species. The exceptional usefulness of this algae for various biochemical and biophysical investigations was the reason for its experimental cultivation in laboratory conditions without the addition of sea-water. There have been numerous investigations on different composition of sea-water review by Hood [9] and Ukeles [18], which indicated a great number of compounds in various concentrations. This refers particularly to organic compounds. Besides sea-water, soil extract has also been introduced to the composition of the medium used so far.

Lateur and Bonotto [10] found that the chemical composition of soil extracts differed for various types of soils. Since it is known that sea-water may contain various amounts of organic compounds and also that the chemical composition of soil extracts is not constant, investigations were carried out to compose a completely artificial medium for the cultivation of Acetabularia. The sea-water was replaced by aqueous solutions containing different salts in various ratios [2, 17]. Provasoli [12] recommended that vitamins be added to the medium instead of the soil extract previously used. It was also demonstrated by Betina [2], that algae cultures on media containing vitamins were less exposed to infections than those cultivated on media with soil extract alone.

This paper presents cultivation conditions of Acetabularia calyculus on artificial media under laboratory conditions.

2. MATERIAL AND METHODS

The original culture of Acetabularia calyculus was taken from the collection of the Timiryazev Institute of Plant Physiology in Moscow in 1976 (gift of prof. Kefeli W.) in a culture medium containing sea-water and ESL and kept for the first period under conditions described by Lateur and Bonotto [10]. After a few weeks, several cells were transferred to conditions described by three variants of artificial culture medium, three various temperatures 22°, 24° and 30°C and two variants of light: a) artificial glow lamp type: Daylight 1269, 40 W with an intensity of about 1300 Lx and b) natural daylight.

Each of the analyzed media contained different salt mixtures enriched with vitamins, as shown in Table 1.

Table 1. Concentration of macroelements and microelements mol/1000 ml in the various artificial culture media

Tab. 1. Stężenie mikroelementów i makroelementów w mol/1000 ml w poszczególnych pożywkach stosowanych w hodowli

Reagent Odczynnik	Medium No. 1 Podłoże 1	Solution Roztwór	Medium No. 2 Podłoże 2	Solution Roztwór	Modified medium No.3 Zmodyfikowane podłoże 3	Solution Roztwór
NaCl	0.4137	A	0.4191	A	0.5710	A
Na_2SO_4	0.0281	A	–		–	
KCl	0.0027	A	0.0093	A	0.0119	A
$NaHCO_3$	0.0023	A	0.0023	A	0.0028	A
KBr	0.0008	A	–		–	
H_3BO_3	0.0004	A	–		–	
$SrCl_3$	0.0001	A	–		–	
NaF	0.00007	A	–		–	
KNO_3	0.0019	A	–		–	
K_2HPO_4	0.00005	A	–		–	
$MgCl_2 \cdot 6H_2O$	0.2470	B	0.0257	A	0.0321	A
$MgSO_4 \cdot 7H_2O$	–		0.0182	A	0.0231	A
$CaCl_2$	0.0090	B	0.0130	B	0.0650	B
Na_2HPO_4	0.0281	D	0.0281	D	0.0281	D
$NaNO_3$	0.2352	D	0.2352	D	0.2352	D
$FeSO_4 \cdot (NH_4)_2 \cdot 6H_2O$	0.0035	C	0.0035	C	0.0035	C
$ZnSO_4 \cdot 7H_2O$	0.0015	C	0.0015	C	0.0015	C
$MnSO_4 \cdot H_2O$	0.0009	C	0.0009	C	0.0009	C
$CuSO_4 \cdot 5H_2O$	0.00012	C	0.00012	C	0.00012	C
$CoSO_4 \cdot 7H_2O$	0.00017	C	0.00017	C	0.00017	C
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.00005	C	0.00005	C	0.00005	C
EDTA-Na	0.0099	C	0.0099	C	0.0099	C

To prepare the media only reagents of pro analysis purity were used.

Each of the described media was composed of four solutions labelled A, B, C and D which were separately sterilized for from 40 to 50 minutes under atmospheric pressure.

After sterilizing and cooling, the particular parts were mixed adding 10 ml of the B, C and D solutions to media Nos. 1 and 2 or adding 62.5 ml of B solution, 12.5 ml of C and 7.5 ml of D solution to medium No. 3.

In all these media the soil extract was replaced by vitamins: 0.3 mg/ /1000 ml of vitamin B_1 and 0.012 mg/1000 ml of B_{12} were added. For further experimental cultivation 300 cells about 1 cm in length were placed in each flask containing 100 ml of medium. Every three weeks the cells were transplanted into a fresh medium where the algae number in the cultivation flasks was simultaneously decreased. 300 cells about 1 - 3 cm in length and from 50 to 70 pieces of over 3 cm were kept in each flask.

3. RESULTS

Summarizing the investigations described, it can be stated that proper medium and light intensity have an important influence on the growth and development of the Acetabularia calyculus cells, while temperature

Table 2. The cap formation of Acetabularia calyculus depending on conditions of culture in artificial media

Tab. 2. Formowanie kapelusza u Acetabularia calyculus w zależności od rodzaju pożywki i warunków hodowli

Cultivation conditions Warunki hodowli		Time necessary for cap formation Czas niezbędny do uformowania kapelusza
Medium No. 1 Podłoże Nr 1		14 months 14 miesięcy
Medium No. 2 Podłoże Nr 2		14 months 14 miesięcy
Medium No. 3 Podłoże Nr 3		5 - 8 months 5 - 8 miesięcy
Illumination 1300 Lx Oświetlenie 1300 Lx		6 - 7 months 6 - 7 miesięcy
Day light Światło dzienne	spring-summer wiosna-lato	5 - 6 months 5 - 6 miesięcy
	autumn-winter jesień zima	7 - 8 months 7 - 8 miesięcy
Temperature 22°C, 24°C Temperatura 22°C, 24°C		no measurable effects efekt niemierzalny
Temperature 30°C Temperatura 30°C		destructive effect efekt destrukcyjny

variations 22° to 24°C, i.e. by ±2°C do not. A temperature of over 30°C has a destructive influence on the cells, causing changes in colour, loss of whorls, or inhibition of formation (Table 2). Acetabularia calyculus cells growing on media Nos. 7 and 2 attained the mature form after the complete biologic cycle in 14 months, but a great number of cells cultivated on these media attained the whorl stage only. Differences between cells cultivated on different media are not visible during the first months of development, but some differences can be observed when Acetabularia calyculus cells form whorls.

Cells cultivated on media Nos. 1 and 2 frequently do not attain the succeeding stadium of development. Acetabularia calyculus growing on modified medium No. 3 goes through all stages of development of its biological cycle. The described strain of Acetabularia calyculus attains mature form after 5 - 8 months, as shown in Table 2.

4. DISCUSSION

An insufficient supply of literature on Acetabularia calyculus cultivation was the reason for choosing the optimum medium used for Acetabularia mediterranea, as the cultivation medium in the experiments described. This resulted from the fact that both species are morphologically and biochemically similar.

The development of Acetabularia mediterranea and Acetabularia calyculus is known to proceed in 13 stages under laboratory conditions. Full and complete growth of Acetabularia mediterranea cells cultivated on media Nos. 1 and 2 can be obtained in 6 - 8 months. Our results demonstrated that the optimal media used for Acetabularia mediterranea were unsuitable for the growth and development of Acetabularia calyculus cells.

Investigations of the optimal media for cultivation of Acetabularia calyculus cells were therefore necessary. After several unsatisfactory results obtained on other media the cultivation of Acetabularia calyculus on modified medium No. 3 was undertaken, where the cells attained all stages of development. The biological cycle of Acetabularia calyculus, however, is slightly longer as compared with Acetabularia mediterranea, taking 9 to 10 months.

Between the 3 - 4 th month Acetabularia calyculus forms whorls on the stalk. The reproductive cap as a generative organ is the next form of development in both cases. The mature cap of Acetabularia calyculus is radial, but its rays are not connected on the periphery as in the case of Acetabularia mediterranea. As the growth and development of the algae on modified medium No. 3 was satisfactory, close analyses and

comparison of all growth stages on media Nos. 1 and 2 were also carried out.

Comparison of the salt concentration of particular media shows that modified medium No. 3 has a higher concentration of macroelements. The modified medium contained more K^+, Na^+, Ca^{++} and Mg^{++} ions. This was based on the knowledge that direct inherence of Na^+ ions in glucose transmission has been observed [11]. On the other hand, the flow of K^+ and Cl^- ions to vacuoles, as well as the outflow of Na^+ cations is possible owing to the transmission systems. These transmission systems depend on light [7]. However, the transmission of K^+ and Cl^- to the cell is more active than that of Na^+. The inherence of Mg^{++} ions on the inner surface of the membrane is imperative for the effective operation of the transmission system. ATP-ase activity is stimulated by K^+ ions on the outer membrane surface and Na^+ ions on the inner surface [11]. It is presumed that these ions are needed to ensure proper activity of Acetabularia calyculus transmission system. The operation of the Cl^- transmission system depends either on the full photosynthetic chain of electron transfer induced by light or the full transmission of electrons in mitochondria operating in the dark [7].

HCO_3^- flow investigations in the cell also indicate the occurrence of bicarbonate pump, owing to which NCO_3^- is assimilated. Therefore, as advised by Puiseux-Dao [14], Lateur and Bonotto [10], exchange of medium every 3 - 4 weeks is necessary. The frequent replacement of media ensures new ion and vitamins proportions and regulates the exchange of gases in the culture.

The influence of light on Acetabularia species has been studied by many authors: Dao [6], Richter [15], Clauss [3, 4, 5], Richter and Kirschtein [16]. Despite the influence on photosynthesis, light intensity can have a direct effect on the growth of algae. It was shown [5], for example, that the stalk growth is promoted by lower light intensity.

Acetabularia calyculus exposed to low light intensity or cultivated in the dark for a certain period is more extended and has a pale green stalk.

When, during cultivation of Acetabularia calyculus, parameters given by Puiseux-Dao [13, 14] were applied, normal cell growth was attained.

Irradiation of cells by sunlight in spring-summer month speeded up cap formation by one month. The caps were larger and greener, whereas daylight alone administered on cells in autumn and winter gave no satisfactory results, the cap being formed, as compared with a light intensity of 1300 Lx, one month later. Algae exposed to this light, form more extended and slightly coloured stalks. It is known that active K^+ and Cl^- ion transmission to cells depends on light, the observed effect may be the result of low light intensity in these months.

It must be kept in mind that it is very important that the optimal

temperature should be determined for vegetative organisms — the most favourable for long-term cultivation, and it is therefore probable Acetabularia species which originates from warm seas grows slowly.

On the other hand according to Betina [2] and Bonotto [1] the optimum temperature for Acetabularia mediterranea is 20 - 22°C. It was found that variations of temperature between 22 - 24°C, i.e. by ±2°C do not influence the cell growth.

In both cases the algae develop steadily and form the reproductive cap. A temperature of 30°C has a destructive influence on the cells, algae exposed to this temperature lose their pigmentation and become pale yellow in 2 -3 weeks. The formation of whorls stops under these conditions. The colour change suggests the pigment variation in the chloroplasts, which affects the photosynthetic process. The small amount of glucose arising in such photosynthetic processes may hinder the plant in forming whorls. Basing on observations and according to our investigations, we have found that in order to receive a new Acetabularia calyculus generation, proper ion concentrations must be maintained in the cultivation medium throughout almost the whole cycle and that the application of proper light intensity is one of the major factors leading to the formation of the reproductive cap.

Agata BARTKIEWICZ, Tadeusz WILCZOK

Śląska Akademia Medyczna
Instytut Chemii i Fizyki — Sosnowiec

HODOWLA ACETABULARIA CALYCULUS NA SZTUCZNEJ POŻYWCE

Streszczenie

Wykazano, że Acetabularia calyculus może być hodowana w całkowicie sztucznych warunkach bez ekstraktu z ziemi i bez naturalnej wody morskiej na następującej pożywce:

A. NaCl — 33.12 g; $MgCl_2 \cdot 6H_2O$ — 6.5 g; $MgSO_4 \cdot 7H_2O$ — 5.7 g; KCl — 0.887 g; $NaHCO_3$ — 0.241 g; woda destylowana — 1000 ml.

B. $CaCl_2$ — 7.15 g; woda destylowana — 1000 ml.

C. $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ — 1.4 g; $ZnSO_4 \cdot 7H_2O$ — 0.44 g; $MnSO_4 \cdot H_2O$ — 0.155 g; $CuSO_4 \cdot 7H_2O$ — 0.031 g; $CoSO_4 \cdot 7H_2O$ — 0.048 g; $(NH_4)_6 \cdot Mo_7 \cdot O_{24} \cdot 4H_2O$ — 0.064 g; EDTA-Na — 3.7 g; woda destylowana — 1000 ml,

D. Na_2HPO_4 — 4 g; $NaNO_3$ — 20 g; woda destylowana — 1000 ml, witaminy B_1 i B_{12}.

Glony hodowano w kolbach Erlenmayera o objętości 500 i 1000 ccm w 22 -

- 24°C w świetle o intensywności 1300 lux. Te warunki pozwalają na normalny rozwój glonów, których cykl biologiczny trwa od 9 do 10 miesięcy.

Rozwój głównych morfotycznych struktur jest następujący: formowanie ryzoidu — 1 miesiąc, wzrost łodygi do 3 miesięcy, formowanie okółków między 4 a 7 miesiącem, formowanie kapelusza 7 - 8 miesiąc, dojrzewanie kapelusza 1 do 2 miesięcy.

REFERENCES

1. Bonotto S., *Some teratological types of morphological differentiation in Acetabularia mediterranea*, Atti. Ass. Genet. Ital., 18, 1973.
2. Betina M. Y., A. A. Yazykov, Y. V Yanushevich, T. N. Zubarev, *Tekhnika kultivirovaniya Acetabularia mediterranea*, Fiziologiya Rastenii, 5, 1973.
3. Clauss H., *Über den Einfluss von Rot und Blaulicht auf das Wachstum kernhaltiger Teile von Acetabularia mediterranea*, Z. Naturforsch., 50, 1963.
4. Clauss H., *Beeinflussung der Morphogenese, Substanzproduktion und Proteinzunahme von Actebaularia mediterranea durch sichtbare Strahlung*, Protoplasma, 65, 1968.
5. Clauss H., *Effect of red and blue light on morphogenesis and metabolism of Acetabularia mediterranea*, in: *Biology of Acetabularia*, New York—London 1970.
6. Dao S. A., *Comportement de l'Acetabularia mediterranea Lam. en culture. Etude de sa croissance*, Rev. Gen. Bot., 61, 1954.
7. Grodziński D. M., *Biofizyka błon komórek roślinnych*, in: *Biofizyka roślin*, Warszawa 1978.
8. Hämmerling J., *Entwicklung und Formbildungsvermögen von Acetabularia mediterranea. I. Die normale Entwicklung*, Biol. Zentralblatt., 51, 1931.
9. Hood D. W. (Ed.), *Symposium on organic matter in natural waters*, Institute of Marine Science, University of Alaska, 1970.
10. Lateur L., S. Bonotto, *Culture of Acetabularia mediterranea in the laboratory*, Bull. Soc, Roy. Bot. Belgique, 106, 1973.
11. Nejfach S. A., *Sprzężenie energii utleniania i transportu przez błony komórkowe*, in: *Mechanizmy integracji przemian komórkowych*, Warszawa 1973.
12. Provasoli L., J. J. A. McLaughlin, M. R. Droop, *The development of artificial media for marine algae*, Arch. Microbiol., 25, 1957.
13. Puiseux-Dao S., *Les Acetabularies, material de la laboratoire*, Ann. Biol., 2, 1963.
14. Puiseux-Dao S., *Acetabularia and cell biology*, London 1979.
15. Richter G., *Die Wirkung von blauer und roter Strahlung auf die Morphogenese von Acetabularia*, Naturwissenschaften, 49, 1962.
16. Richter G., M. J. Kirschtein, *Regeneration und Photosynthese-Leistung kernhaltiger Zell-Teilstücke von Acetabularia in blauer und roter Strahlung*, Z. Planzenphysiol., 54, 1966.
17. Shephard D. C., *Axenic culture of Acetabularia in a synthetic medium*, in: *Methods in cell physiology* (Prescott D., Ed.), New York and London, Vol. 4, 1970.
18. Ukeles R., W. E. Rose, *Observation on organic carbon utilization by photosynthetic marine microalgae*, Mar. Biol., 37, 1976.