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NOTES ON THE METHOD FOR PREPARING BACTERIA-FREE CULTURES OF GREEN ALGAE BY ULTRA-VIOLET IRRADIATION

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Among the various methods which were thus far employed to purify algal cultures from bacteria (FOGG 1942, PRINGSHEIM 1946, ALLEN 1952, WAI 1955, KRAUSS 1958 etc.) the ultra-violet irradiation is one of the most simple procedures, and the sufficiency of a very small quantity of algal material needed in this treatment means also an advantage over other methods.

Ultra-violet irradiation was first used for the purification of blue-green algae. To free these algae from bacteria is always a matter of some difficulty because of the gelatinous sheaths with which they are generally surrounded, and their purification was eventually achieved by treatment with ultra-violet light, since a suitable irradiation kills contaminating bacteria without damaging the algae (ALLISON and MORRIS 1930, GERLOFF, FITZGERALD and SKOOG 1950, FOGG and WOLFE 1954, KRAUSS 1958). Only ALLEN (1952) has pointed out the desirability of avoiding "mutagenic agents" (antibiotica, ultra-violet light etc.) in obtaining pure cultures and employes, instead, repeated transfers from the peripheries of spreading colonies.

In order to provide material for the physiological studies of algae recently started in our laboratories, large number of various bacteria-free algal strains had to be isolated. Beside other procedures irradiation was also used to kill the adhering bacteria.

Species-pure or so-called uni-algal cultures were obtained by the usual bacteriological techniques of streaking diluted lake water or mixed algal suspensions on 1.5 per cent agar plates prepared of Knop-Pringsheim nutrient solution (PRINGSHEIM 1946, 35). From the surface of the agar plates the desired organisms were selected and subcultures prepared from them. By repeated subculturing, unialgal cultures were obtained, which were transferred into test tubes on agar slants containing only inorganic salts.

For ultra-violet irradiation a small quantity of algae was suspended into a liquid nutrient solution from these "inorganic" agar slants. This algal suspension was poured into a quartz beaker and irradiated for ten minutes with quartz-jacketed "Hanau" mercury vapor lamp (Typ. S. 300) from 30 cm distance. The suspension was agitated by continuous stirring during the irradiation period. Samples were taken with sterile pipettes from the beaker at ½, 1, 2, 4, 6, and 10 minute intervals. Various dilution cultures were made of each on a large number of agar plates containing nutrient substances as follows :

0,500 g potassium nitrate
0,100 g magnesium sulfate cryst.
0,100 g calcium nitrate
0,200 g dipotassium phosphate
0,002 g ferric citrate
1 ml HOAGLAND's A—Z solution
5 g glucose
2 g peptone
2 ml yeast hydrolysate
15 g washed agar-agar
1000 ml glass distilled water

Bacteria, fungi or molds easily develop on this glucose-peptone medium but sufficient irradiation — depending on the delicacy of the different strains — and a large number of dilutions in most cases result plates on which the number of bacterial colonies is restricted so that separate colonies of algae can be easily transferred to "organic" agar slants under sterile conditions.

The strains thus purified were further checked for purity on three agar slants of different composition: in the above-mentioned glucose-peptone agar (pH 7), on bouillon-agar (pH 7) (FEHÉR 1944, 168) and on FEHÉR's organic agar (pH 4) for fungi or molds (FEHÉR 1944, 192).

Bacteria-free strains are kept on glucose-peptone agar slants and are transferred to fresh slants once in every three months.

It is well known that algae living on media containing glucose may undergo physiological and morphological changes but when they are transferred to inorganic nutrient solution in most cases they become similar to the starting forms.

The morphological identity of untreated and ultra-violet irradiated cultures of eight *Scenedesmus* strains grown in liquid inorganic media was studied and no morphological changes, induced by ultra-violet light, could be found.

For comparison of physiological properties, the photosynthetic power of ultra-violet irradiated (bacteria-free) and untreated portions of 172. *Kirchneriella contorta* (SCHMIDLE) BOHLIN strain was measured by the usual Warburg method (WARBURG 1918, GAFFRON 1939), at 25° C and *c.* 5000 lux. The values of photosynthetic rate in three different media, expressed as μl O_2/1 mg dry matter/1 hour are as follows:

	Untreated	UV-irradiated for 6 minutes
K_2CO_3 3 mmol/litre	32,6	29,2
$KHCO_3$ 3 mmol/litre without free CO_2	42,0	42,2
$KHCO_3$ 3 mmol/litre + free CO_2	46,4	49,2

The results show that the ultra-violet irradiation caused no injurious changes in photosynthetic activity of this strain.

From the collection of the Hungarian Biological Research Institute the following strains were purified by this method:

Strain No.	Species
1329	*Chlorosphaera angulosa* (CORDA) KLEBS
25	*Cystococcus humicola* NAEG.
4066	*Pediastrum boryanum* (TURP.) MENEGH.
4072	*Coelastrum microporum* NAEG.
2257	*Coelastrum sp.*
634	*Chlorella pyrenoidosa* CHICK.
1896	*C. pyrenoidosa* CHICK.
2535	*C. pyrenoidosa* CHICK.
4077	*C. pyrenoidosa* CHICK.
3499	*Chlorella sp.*
638	*?Tetracoccus botryoides* W. WEST.
4068	*Ankistrodesmus sp.*
172	*Kirchneriella contorta* (SCHMIDLE) BOHLIN
4079	*Scenedesmus acuminatus* (LAGERH.) CHOD.
2543	*S. acutus* MEY. *f. alternans* HORTOB.
4073	*S. armatus* CHOD.
4063	*S. armatus* CHOD. *var. boglárensis* HORTOB.
198	*S. quadricauda* (TURP.) BRÉB.
4061	*S. quadricauda* (TURP.) BRÉB.
4070	*S. spinosus* CHOD.
4069	*S. tenuispina* CHOD.
4059	*Scenedesmus sp.*
4177	*Scenedesmus sp.*
1883	*Heterococcus ?chodati* VISCHER
1898	*H. moniliformis* VISCHER

And other eleven unidentified strains (two diatomes).

In our laboratory attempts were made to simplify the process of isolation and purification. For this purpose not suspensions of unialgal cultures but natural populations of algae collected with a plankton net were directly irradiated with ultra-violet light and treated in general as described above. In this way numerous pure strains can be isolated simultaneously from the bacteria-free colonies of different species developing on the surface of "organic" agar plates. The isolated strains were checked for purity in the usual manner, and they were determined after having been transferred into inorganic liquid media. However, this simplified procedure gave good results only in algal suspensions collected from water bodies showing a low number of bacteria, *e. g.* the collection has to be made in winter or at late autumn.

On one occasion eight different strains were isolated with this "direct" method from Lake Balaton and on another occasion nine strains from the pond "Belső-tó" at Tihany.

Summary

Species-pure algal cultures were irradiated with ultra-violet light in order to purify them from bacteria. The suspensions thus irradiated were plated on agar plates containing glucose, peptone and yeast hydrolysate besides the usual inorganic substances (Knop-Pringsheim solution).

Good results were also obtained when irradiating natural net plankton samples.

In morphological and physiological properties of the treated algal strains no changes were observed in comparison to the untreated ones.

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IBOLYÁNTÚLI SUGÁRZÁS HASZNÁLATA ZÖLD ALGA-TENYÉSZETEK BAKTÉRIUM MENTESÍTÉSÉRE

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Összefoglalás

Az alga-kultúrák baktérium-mentesítésének módszerei közül az ibolyántúli fény besugárzása a legegyszerűbb és nagy előnye, hogy egészen kis mennyiségű algaszuszpenzióval is keresztülvihető.

A bakteriológiában szokásos szélesztéses eljárással „szervetlen" ágár lemezeken egy fajból álló tenyészeteket állítunk elő, melyekben szerves táplálék hiányában a baktérium és penész szennyeződés nem hatalmasodhat el. Ebből az ún. egy-faj tenyészetből steril tápoldatban szuszpenziót készítünk. Az UV-besugárzást kvarc edényben, állandó kevergetés közben „Hanau" kvarclámpával végezzük 10 percig. 1/2, 1, 2, 4, 6 és 10 perc múlva mintákat veszünk a szuszpenzióból steril pipettával és nagyszámú terítést készítünk különböző hígítással „szerves" (szőlőcukor és pepton-tartalmú) ágár lemezen. Ennek összetételét lásd 344. o. Ezekre a lemezekre is jutnak még élő baktériumok és gombák,

de a besugárzás és a hígítás fokától függően mindig kapunk olyan lemezt, melyen izolált, baktériummentes algatelepek is fejlődnek. Ezeket kémcsőbe visszük „szerves" ferde ágárra, steril körülmények között. Az esetleges fertőződés a szerves ágáron azonnal jelentkezik. A törzsek tisztaságát különféle összetételű és pH-jú táptalajokon tenyésztve vizsgáljuk meg.

Az ibolyántúli fénnyel baktérium mentesített algákon morfológiai változást nem tapasztaltunk, sőt egy *Kirchneriella* törzsünkön végzett fotoszintézis mérés szerint az UV-kezelés élettani változást sem okozott.

A 345. oldalon soroljuk fel tenyészet-gyűjteményünk ibolyántúli fénnyel baktérium-mentesített törzseit.

Külön ki kell térnünk egy egyszerűsített eljárásunkra, melynek segítségével az izolálás és baktérium-mentesítés egyidejűleg elvégezhető. Ha a természetes vízből származó planktonmintát közvetlenül sugározzuk be — egyébként a fent leírt eljárással — a baktériumok és gombák számát annyira visszaszoríthatjuk, hogy nagyszámú faj baktérium-mentes törzsét izolálhatjuk, elkerülve az egy-faj tenyészetek előállítását.

ПРИМЕНЕНИЕ УЛЬТРАФИОЛЕТОВОГО ОБЛУЧЕНИЯ ДЛЯ СПАСЕНИЯ КУЛЬТУР ЗЕЛЕНЫХ ВОДОРОСЛЕЙ ОТ БАКТЕРИЙ

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Резюме

Из методов освобождения культур водорослей от бактерий, самым простым способом является облучение ультрафиолетовым светом, обладающим тем преимуществом, что его можно осуществить и с помощью совсем малого количества суспензии водорослей.

При помощи обычного в бактериологии способа рассева, на «неорганических» агаровых пластинках разводятся состоящие из одного вида культуры, в которых, за неимением органического питания, загрязнение бактериями и плесенью не может распространяться. Из этой т. н. одновидовой культуры в стерильном питательном растворе изготовляется суспензия. Облучения ультрафиолетовым светом производится в кварцовом сосуде, при постоянном перемешивании, с помощью кварцевой лампы типа «Ганау», в течение 10 минут. Через $^1/_2$—1—2—4—6 и 10 минут, при помощи стерильной пипетки, берут пробы из суспензии и в различных разбавлениях, на «органической» (содержащей глюкозу и пептон) агаровой пластинке изготовляют многочисленные стланья. Состав пластинки см. на стр. ... И на этих пластинках наблюдаются еще живые бактерии и грибы, однако, в зависимости от степени облучения и разбавления, всегда получается пластинка, на которой развиваются также и изолированные, свободные от бактерий колонии водорослей. Эти колонии переносят в пробирку на «органический» косой агар, при стерильных условиях. Возможное заражение немедленно проявляется на органическом агаре. Чистота штаммов проверяется при культивировании на питательных средах различного состава и различного значения pH.

У водорослей, освобожденных от бактерий с помощью ультрафиолетового света, морфологические изменения не наблюдались, да и по измерению фотосинтеза, исполненного на одном штамме Kirchneriella ультрафиолетовое облучение даже физиологического изменения не вызывало.

На стр. .... приводятся штаммы подбора культур, освобожденные от бактерий путем ультрафиолетового облучения.

Особенно следует отметить упрощенный способ, с помощью которого изолирование и освобождение от бактерий исполняются одновременно. Если происходящая из естественной воды проба планктона облучается непосредственно — в остальном по вышеуказанному способу —, то количество бактерий и грибов уменьшается до такой степени, что удается изолировать свободные от бактерий штаммы многочисленных видов, избегая при этом создание одновидовых культур.