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Influence of Low Doses of Mutagens on Growth of Algae on a Solid Medium

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Abstract. The effects of low doses of two mutagens (UV-light and ethyl methansulphonate) on the growth of algae on a solid minimal (anorganic) medium were studied. It was supposed that low doses of mutagens will be more suitable for the induction of growth mutations.

The UV-light had an inhibitory effect in the whole range of the applied doses on the growth of colonies of algae from the cells inoculated on the solid medium immediately after irradiation.

Ethyl methansulphonate produced growth stimulation if applied in the lowest doses. The growth was inhibited in a further range of doses and then there appeared the range of lethal doses.

The growth responses to the influence of these mutagens were different in all the three algal species used and to the previous cultivation conditions before their exposure to the mutagens. It is certain that most of these growth responses are only modifications.

The influence of ethyl methansulphonate differed according to the method of application. If it affected the algae for a long time (it means if they were inoculated directly on the solid medium containing mutagens), or, if the algae were exposed to its influence immediately before their inoculation on the solid medium, the growth responses of colonies were quite different.

Growth responses with the single studied strains differed quantitatively only.

The selections of algae on a solid medium can be carried out according to the characters of single cells or according to the character of their organizationally bound groups (e.g. coenobia) immediately after the inoculation. Later, the selections are carried out according to the growth intensity and features of colonies from single cells (coenobia). The characteristic growth intensity and features of the colonies on a solid medium (and, consequently of the cells compounding them) are given under standard cultivation conditions partly by the genetical constitution of the strains used, partly by the modification influences affecting all the colonies either in the same way (macroclimate of the cultivation dish), or, sometimes in a different way (according to the submicroclimate on the surface of the medium). In the scope of one strain the colonies variability is also conditioned by the individuality of single cells (coenobia) and their ontogenetical phase (of course,

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it does not concern the material from synchronous culture). It is very important, especially with the coenobial algae such as *Scenedesmus quadricauda*, if all the four cells in the coenobium share equally, with their progenies, in the growth of the colony.

All these influences manifest themselves by the variability in the size and features of the colonies grown from single cells (coenobia). It is necessary to take this variability into account when selecting on the solid medium. Among other factors, which can enlarge or narrow the variability in the size of colonies, or perhaps shift it both in negative and positive directions, various mutagens play an important role. From this point of view I have investigated the influence of both, physical (UV-irradiation) and chemical (ethyl methansulphonate) mutagens in this work.

Material and Methods

Material. For the experiments the following algal strains were chosen: the coenobial chlorococcal alga *Scenedesmus quadricauda* (TURP.) BRÉB., strain Greifswald 15, unicelled chlorococcal alga *Chlorella vulgaris* BEIJ., var. *vulgaris* strain Sorokin-Myers Tx 71105, both these strains were from the collection of the Laboratory of Experimental Algology of the Microbiological Institute of the Czechoslovak Academy of Sciences, Třeboň. Further, the flagellate *Chlamydomonas reinhardi* strain A20 was from the Collection of Autotrophic Organisms of the Czechoslovak Academy of Sciences, Prague. Cultures, taken for experiments from the solid medium, were not older than 30 days after inoculation. Cultures from the liquid medium were taken from the test-tube cultivation apparatus (NEČAS, 1966) where they were cultivated approximately in the linear phase of the growth curve, in a temperature of 25—30° C, with illumination 0·056 cal/cm square . min. PhAR. The medium was the same as that used for the preparation of the solid medium.

Methods. A special irradiation lamp was used equipped with two germicidal tubes Phillips TUV 15 W L4 constructed in our laboratory. UV-radiation passes through the Schott UG5 filter. In this way the narrowed spectrum is gained which corresponds better with the absorption region of DNA. The irradiated layer of the suspension in the Petri dish without cover was 1 mm thick. The distance of the light source of the suspension surface was 34 cm. The irradiation intervals are given with the individual experiments. The density of the radiation flux was 7.1 μA to 1 cm square (measured by a dosimeter, constructed in our laboratory and calibrated according to the standard of Dr. Věchet, Microbiological Institute of the Czechoslovak Academy of Sciences).

For the studies of the ethyl methansulphonate (EMS) effects the substance (prepared in the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences, obtained by the courtesy of Dr. Fučík) was used in dilutions, given with each single experiment. The EMS was applied in the same doses partly to the solid medium, partly for a short time

into the liquid medium before inoculating on the solid medium (the times of its action are given with individual experiments). The cultures with EMS in the liquid medium were darkened in the course of its action with aluminium foil in order to avoid its decomposition in the strong light. Before inoculation the suspension in this liquid medium on the solid medium the cells (coenobia) were not deprived of the imperceptible amounts of the EMS either by centrifugation or by washing.

As a solid cultivation medium a 2% agar with 1/4 concentration of the medium according to BJÖRKMAN et al. (1955) modified by KOMÁREK et RŮŽIČKA (1968) was used. The control cultures and the ones affected by EMS were inoculated on the dry surface of the solid agar in Petri dishes ($\varnothing = 9$ cm) by means of a revolving inoculating device. The inoculated cultures in the dishes (both control as well as experimental variants), were darkened for 24 h in order to prevent photoreactivation of cells irradiated by UV-light and the premature decomposition of EMS. Then the cultures were exposed to the continuous illumination of fluorescent tubes Tesla Z25WB. The dishes with the cultures were placed under two different light intensities, namely 1350 lux (0·021 cal.cm^{-2} min^{-1} PhAR) and 750 lux (0·015 cal.cm^{-2} min^{-1} PhAR), measured in the middle of the dish covers. The density of the inoculum was such as to ensure the isolation of most of the colonies and that these colonies came from one cell.

The growth of colonies was measured directly on the surface of the agar with objective 10 and measuring ocular 8. The size of the colonies was expressed by the diameter of the circle outline of the growing colony. The growing colonies of *Chlorella* and *Chlamydomonas* are regularly circular in shape. The margins of *Scenedesmus* colonies are slightly irregular (coenobial character), however they are circular in shape, too. Therefore I have measured only the diameter of the colonies outlines including the surface of the medium covered entirely with the cells.

50 (in some experiments only 25) colonies from each dish taken at random were measured. Only such colonies were excluded as those of abnormal size caused by the well-known interfering effect. The colonies of the coenobial alga *Scenedesmus* had to meet, first of all, the requirement that they originated from the whole coenobium (that means from all four cells). The average values were estimated from all the measured values by the usual statistical method. According to the data obtained, the growth curves of the average colonies were designated for the control and experimental variants in single experiments.

Results and Discussion

Presuppositions for the evaluation of the growth of algal colonies on solid medium.

On the basis of their experiments, some authors (WETHERELL and KRAUS 1957; HARTSHORNE 1955, EVERSOLE 1956; TSCHÄPE 1966 and others) have shown, that it is possible to isolate the growth mutants on the solid medium according to the colonies growth from cells, inoculated there after

their being influenced by mutagens. KVITKO 1961, using a mixture of different coloured strains of *Chlorella* proved, that when carefully inoculated on the surface of a solid medium colonies originating from two cells occurred only seldom. Although *Scenedesmus quadricanda* has not such favourable characters for a good and easy inoculation it is possible, with a little practice, to distinguish colonies which originated from one coenobium from those which originated from two. The later must be excluded from the measurement. It is rather difficult to exclude colonies originating from one coenobium only when some of its cells have died. After some experience even this is possible.

The probability of a successful selection of growth mutants on the minimal medium decreases by the higher frequencies of modifications, above all, those which are due to the specific effects of the used mutagens. The effects of the mutagens are mostly studied in doses, which fall in the range of the efficacious mutagenesis and manifest themselves by conspicuous lasting variations. Less attention was paid to the range of lower doses in algae.

It was shown, in our previous experiments (NEČAS 1966), that it is necessary to take into consideration even the effects of the lower mutagene doses on the growth of colonies from the influenced cells or coenobia when selecting the growth mutants. This has been proved by the results of TSCHÄPE (1966) and NEČÁSEK et al. (1967) with bacteria. There exists, among the surviving cells a certain number which show the effects of the lower doses of mutagene without producing any changes of mutation character, only the modification ones. When selecting, it is very difficult to distinguish them on the solid medium from the persistent changes, in which we are particularly interested.

In order to give a clear picture, the range of variability in the single marked points is not presented in the figures of the growth curves. The curves are drawn in the most probable way ensuring from the comparison of results of other experiments carried out with the same mutagens doses. The range of the variability of individual values around the diameter, pointed out in

Fig. 1

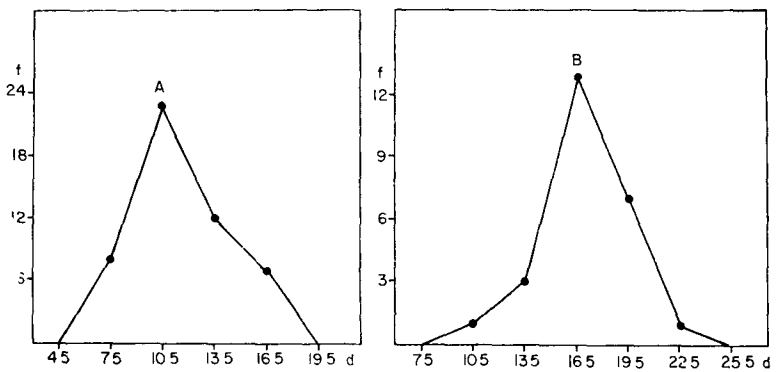

Fig. 1. The distribution curves of the colonies size of *Scenedesmus quadricauda* (A) and *Chlorella vulgaris* (B) in one point of the growth curve.
A n = 50 B n = 25
d = size of colonies in micrometric ocular sections
f = frequencies of colonies of the given sizes

the growth curves follows from the comparison of the distribution curves concerning the colonies growth for *Scenedesmus* and *Chlorella* (Fig. 1). (It corresponds with *Chlamydomonas*, too.) The growth curves of the control variant of one experiment are given from both levels of light intensities in Fig. 2. Both curves are significantly different. They prove the reliability of results presented in different variants if they are in accordance in both illumination levels. From the comparison of the responses in both illumination levels the orientation information concerning the interaction of the influencing factor with illumination can be obtained.

Fig. 2.

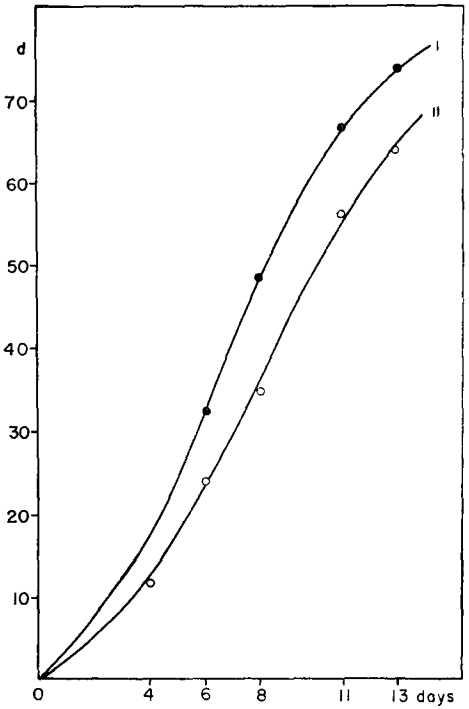

Fig. 3.

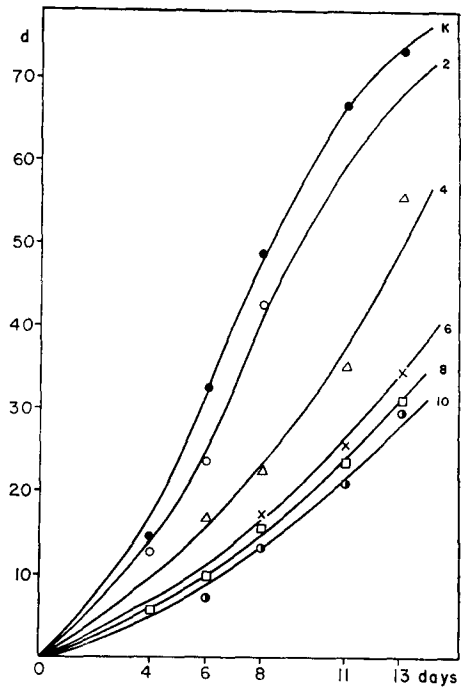

Fig. 2. The growth curves of the *Scenedesmus quadricauda* colonies on minimal (anorganic) solid medium in the cultivation shelf with continual illumination of 1350 lux (0.021 cal/cm² min. PhAR) (I) and 750 lux (0.015 cal.cm² . min^{-1} PhAR.) (II). The culture was taken from the solid medium. The size of colonies in single points of the growth curves is expressed by the averages estimated from the distribution curves (fig. 1) in the sections of the micrometrical ocular measure (1 section (d) = 13,46 μ). It holds for the other mentioned figures, too.

Fig. 3. The growth curves of the *Scenedesmus quadricauda* colonies on the minimal solid medium with continual illumination of 1350 lux after irradiation with UV-light. The culture was taken from the solid medium.
K = control; 2, 4, 6, 8, 10 = variants of the experiments according to the time of irradiation with UV-light in minutes with radiation flux 7·1 μA to 1 cm².

Inhibition of the growth of algal colonies after irradiation with UV-light.

As it is given in Figure 3. the inhibition of the growth of algal colonies of *Scenedesmus quadricauda* manifested itself after irradiation with UV-light at all applied doses, even at low ones.

It means that when selecting the colonies according to their growth on the solid medium after the pretreatment with this mutagen, it is necessary to take into account the raised frequencies of the negative modifications. A better growth of colony which does not exceed the level of the control variant can be caused by the slighter mutagen influence. Such colonies, which start growing very rapidly above the control level after the short initial inhibition, can offer a certain selection perspective. However the decisive criterion remains the testing in the liquid medium.

Fig. 4.

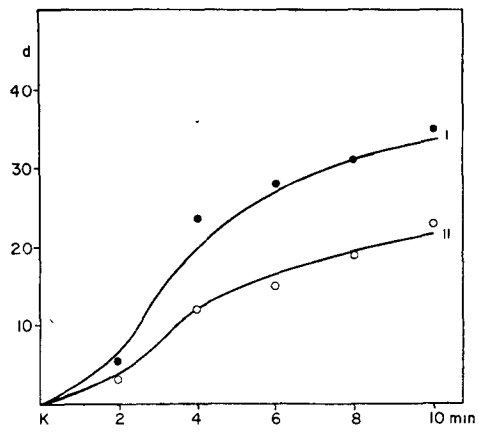

Fig. 5.

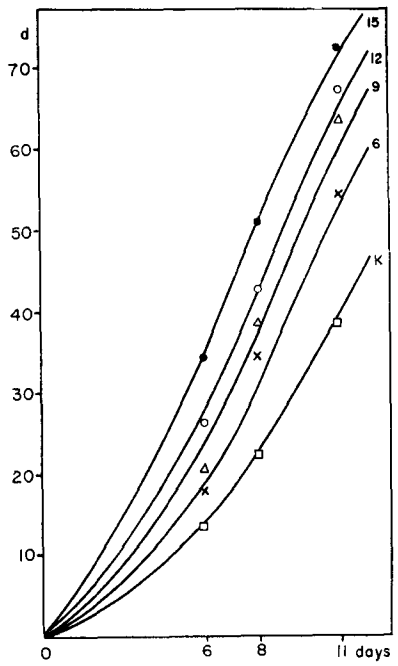

Fig. 4. Dependence of the inhibition of the colonies growth of *Scenedesmus quadricauda* on the minimal solid medium upon the irradiation doses of UV-light, expressed as decrease of the colonies size as compared with the control in the sections of micrometrical ocular measure after 12 days of growth. The cultures were taken from the solid medium.
I = experiment in the figure 3,
II = replication of the same experiment with other material of the same strain,
d = size of colonies in the control minus size of colonies in the variant.

Fig. 5. The growth curves of the *Chlorella vulgaris* colonies on the minimal solid medium in the cultivation shelf with continual illumination of 750 lux. The respective doses of EMS were added to the solid medium.
The culture was taken from the cultivation apparatus from the liquid medium.
K = control; 6, 9, 12, 15 = experimental variants according to the doses of EMS in $x \cdot 10^{-3}$ ml of EMS/25 ml of medium.

It follows from the comparison of the dependence curves of the inhibition growth effect on the UV-light doses in both illumination levels (Fig. 4) that the single experiments do not offer quantitatively reproducible results. The shape of the curves does not change very much even in their relatively different position. It proves, that not only the interaction with the cultivation factors on solid medium plays an important role here, first of all the illumination, but it depends also on the conditions under which the culture grew before being influenced by the mutagen. There is a very important difference between the culture growing on a solid medium and this one growing in a liquid medium before irradiation with UV-light. The cultures from the solid medium, if they are not very old, are usually more resistant (NEČAS, unpublished yet).

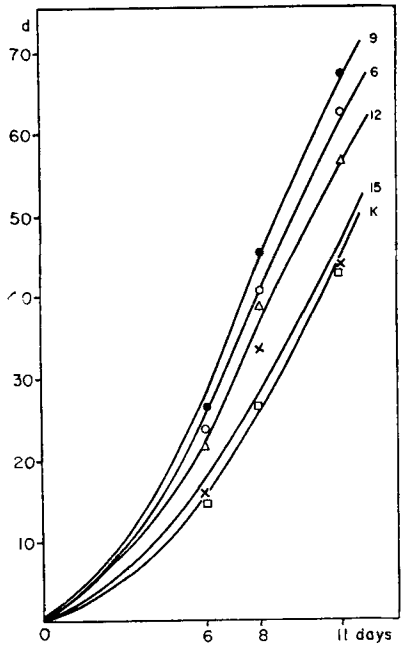

Fig. 6.

Fig. 7.

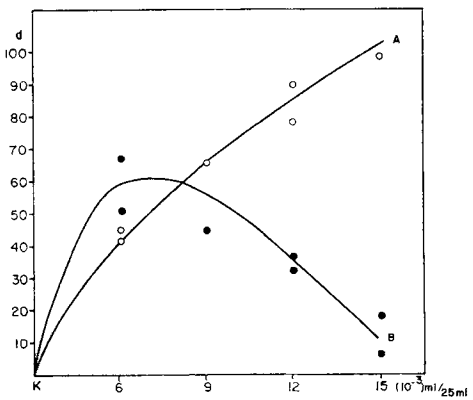

Fig. 6. The growth curves of the *Chlorella vulgaris* colonies on the minimal solid medium in the cultivation shelf with continual illumination 1350 lux after the inoculation of the culture. This culture was exposed before inoculating for 4 hours in the aerated liquid medium in darkness with the respective doses of EMS. The culture was taken from the cultivation apparatus from the liquid medium.
K = control; 6, 9, 12, 15 = experimental variants according to the doses of EMS in x . 10^{-3} ml of EMS/25 ml of medium.

Fig. 7. The dependence of the growth stimulation of *Chlorella vulgaris* colonies on the minimal solid medium on the EMS doses, expressed as increase of the colonies size as compared with the control in the sections of the micrometrical ocular measure after 12 days of growth. The cultures were taken from the cultivation apparatus from the liquid medium.
A = EMS was added to the solid medium; B = EMS was added to the liquid medium for 4 hours before the inoculation on the solid medium.
d = size of colonies in the variant minus size of colonies in the control.

The mechanism of the growth inhibition especially the question if it is more affected, by the proper growth of single cells or by the whole process of the cell division will be the subject of our further studies.

Stimulation of the growth of algal colonies by ethyl methansulphonate.

The chemical mutagen ethyl methansulphonate (EMS) caused, in all low doses with all the used algal strains, a contrary effect on the growth of colonies than the UV-light. The stimulation in the colonies growth took place here but its dependence on the doses was different according to the kind of application. Given in Fig. 5 there are the growth curves of the colonies of *Chlorella vulgaris* on solid agar, which was enriched by various doses of EMS. All the applied doses showed growth stimulation, the higher were the doses the higher the stimulation. When applying the same doses to the darkened culture in the liquid medium for 4 hours before inoculating on a solid medium. EMS stimulates the growth of colonies, too (Fig. 6), but the dependence of the effect on the doses is, in this case, quite different.

The comparison of the dependence of growth responses of colonies on the EMS doses, applied according to the first or second method of application is given in Fig. 7. It is very difficult to explain these dependency differences

Fig. 8

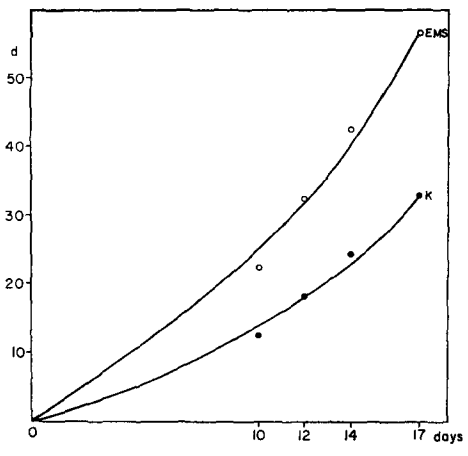

Fig. 9

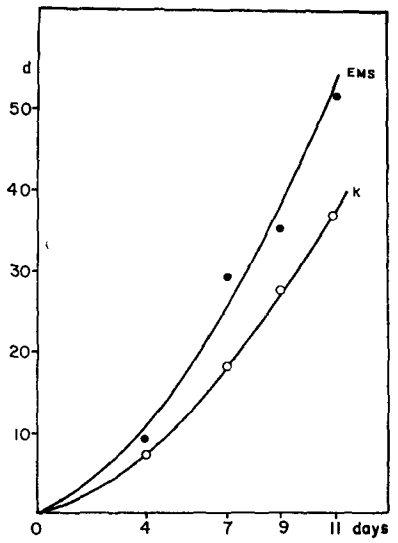

Fig. 8. The growth curves *Chlamydomonas reinhardi* colonies on the minimal solid medium in the cultivation shelf with continual illumination 1350 lux. The culture was taken from the solid medium. K = control; EMS = experimental variant with 9.10^{-3} ml of EMS/ /25 ml of medium.

Fig. 9. The growth curves of *Scenedesmus quadricauda* colonies on the minimal solid medium in the cultivation shelf under the continual illumination 1350 lux. The culture was taken from the cultivation apparatus from the liquid medium.
K = control; EMS = experimental variant with 9.10^{-3} ml of EMS/25 ml of medium.

to any extent. It has not yet been determined the time in which, under given cultivation conditions, the EMS stays in the solid medium in the form of the original substance, its decomposition, if any, and if it can be metabolized in lower doses by the algae. When applying slightly increased doses no re-activation of the culture (not even after any length of time) takes place. Therefore it does not seem that any eventual quick decomposition under given conditions to harmless or stimulation products occur. For these reasons it can be supposed that it is a question of the prolonged influence of the original

Fig. 10.

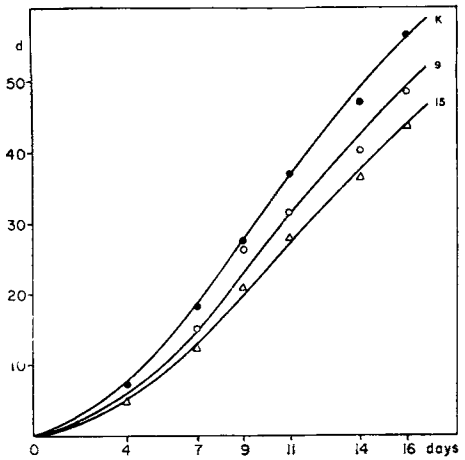

Fig. 10. The growth curves of *Chlorella vulgaris* colonies on the minimal solid medium in the cultivation shelf under the continual illumination 750 lux. The culture was cultivated before the inoculation in the aerated liquid medium for 22 hours in darkness with the respective doses of EMS. The culture was taken from the cultivation apparatus from the liquid medium.
K = control; 9, 15 = experimental variants according to the doses of EMS in x . 10^{-3} ml of EMS/25 ml of medium.

substance in the sublethal level. Fig. 10 verifies this fact to a certain degree; it shows that a longer stay of the algal cells maintained in a liquid medium in darkness with the addition of EMS doses graduated in the same way as in the previous experiments produces even after 22 h the growth inhibition proportional to the doses. The transition from the stimulation range to the inhibition one, when applying EMS to the liquid medium, is clearly visible, however on the solid medium it is more enhanced. When comparing Fig. 6 with Fig. 10 it can be seen that the dosage of EMS and the time of application in the liquid medium causes different growth effects with colonies, growing from the influenced cells on the solid medium. On the whole, it is possible to say that the cultures taken from the solid medium immediately before coming under influence of EMS are more resistant and they respond less sensitively to the graduated doses. In the course of further studies of the growth effects of EMS it will be necessary to find out its influence on the proper growth of cells and in the process of cell division in order to explain the mechanism of its stimulative and inhibitory effects.

The difference in the tolerance and lethality levels of various physical and chemical mutagens in forms differing only little from the taxonomical point of view is known. It can be seen from the comparison of figures 5, 6, 8 and 9 that there also exist considerable differences in the responses of our three species to EMS. Therefore, as mentioned above, these differences must be considered not only from the taxonomical point of view but also it is necessary to pay attention to the previous cultivation conditions of the culture.

Conclusion

It is clear from the results of this study that when the clonal algal strains are selected from the surface of the solid medium, originating from populations of cells affected by mutagens, it is necessary to take into consideration their specific modification effects on the growth of algal colonies. When selecting the growth mutants under these conditions, only such mutants should be chosen that have an undamaged photosynthetical apparatus with the photosynthetical process passing normally, but which have changed in other features or characters important from the production point of view. They exert on the minimal medium a normal or a more intensive growth than the initial strain. It was discovered with both studied mutagens that they have modification effects on the colonies growth on the solid medium. The UV-light produces the inhibitory effects in the whole range of the values used. At low doses of EMS the transition from the stimulative growth effects to the inhibitory ones manifest itself with its increasing doses.

The modification effects of mutagens to the colonies growth lower the probability of the successful selection of growth mutations already on the solid medium. The more successful these selections are, the narrower is the range of sets of selected strains for the decisive productivity testing in liquid medium, which is very laborious and time consuming (RŮŽIČKA and SIMMER 1968).

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V této práci byly studovány účinky nízkých dózí dvou mutagenů (UV-světla a ethylmethansulfonátu) na růst řas na pevném minimálním (anorganickém) mediu.

UV-světlo vykazovalo v celé oblasti aplikovaných hodnot inhibiční účinky na růst kolonií řas, naočkovaných na pevné medium bezprostředně po ozáření.

Ethylmethansulfonát působil v nízkých dávkách nejdříve stimulaci růstu pak v určité úzké oblasti dávek inhiboval růst a za oblastí dávek inhibujících růst přišla náhle oblast dávek letálních. Charakter růstové reakce na působení těchto mutagenů byl ve značné míře určován u jednotlivých druhů řas předcházejícími podmínkami, ve kterých rostla kultura před ovlivněním mutagenem. U ethylmethansulfonátu bylo působení rozdílné také podle toho, zda působil chronicky (byl aplikován přímo do pevného media) nebo zda se jím působilo na řasy akutně (před jejich naočkováním na pevné medium). Růstové reakce u jednotlivých studovaných druhů řas byly jen kvantitativně odlišné.

Й. НЕЧАС, Альгологическая лаборатория микробиологического института ЧСАН, Тржебонь: **Рост водорослей на плотной среде после воздействия низкими дозами мутагенов.** — Biol. Plant. 10 : 374—384, 1968.

В настоящей работе исследовалось действие низких доз двух мутагенов (УФ света и этильметансульфоната) на рост водорослей на плотной минимальной (неорганической среде. УФ свет имел во всей области примененных значений подавляющее действие на рост колоний водорослей, перенесенных на плотную среду непосредственно после облучения. Этильметансульфонат в низких дозах сперва стимулировал рост, в определенной узкой области подавлял рост после чего наступила область летальных доз. Характер ростовой реакции на воздействие этих мутагенов в значительной мере определялся у отдельных видов водорослей условиями роста культуры перед воздействием мутагенами. В случае этильметансульфоната результат также зависел от способа применения — хронически (прибавлен в плотную среду) или импульсно (перед пересадкой на плотную среду). Ростовые реакции у отдельных изученых видов водорослей отличались лишь в количественном отношении.