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Selection and application of antibiotics for the removal of fungal and bacterial contamination in algal cultures

Výběr a použití antibiotik při odstranění houbové a bakteriální kontaminace řasových kultur

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Bednářová M.[1]), T. Kalina[2]) and V. Šašek[3]) (1976): Selection and application of antibiotics for the removal of fungal and bacterial contamination in algal cultures. — Preslia, Praha, 48 : 259—272.

The authors follow the removal of fungal contaminants in algal cultures by nystatin, actidione, pimaricin and mucidin. Effects of these antibiotics were tested against eight fungal species which were isolated from the algal cultures. The best results were obtained when mucidin or pimaricin was applied. The concentration of antibiotics which did not affect the algae but limited the fungal growth was estimated. The suitable concentration of mucidin was 2 μg/ml and that of pimaricin 10 μg/ml. The retardment of fungal contamination often resulted in rapid growth of bacterial contaminants. Bacteria were usually removed by ampicillin in concentration of 1000 μg/ml. The routine method for purification of the algal cultures is described and discussed.

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INTRODUCTION

A serious problem in laboratory cultivation of algae is the obtaining and maintenance of the pure axenic cultures. Such cultures are necessary not only for precise biochemical, physiological or genetic studies but also for the observation of morphology, development and the utrastructure of algae.

Many ways of algal cultures purification have been described. The old methods which are the most delicate from the biological point of view (thin smears, dilution, washing and others) are successful only to a certain extent. The mechanical purification is promoted by the relatively complicated application of ultrasound (Brown et Bishoff 1962). The germicidal UV light shows a mutagenic effect (Nečas 1973). The treatment by different bacteriostatic substances (e.g. potassium telurite, benzalconicum chloride and bengal rose) has only a limited utility.

Since 1951 many experiments with application of different antibiotics in algal cultures axenisation have been made (Provasoli et al. 1951, Droop 1967, Jones et al. 1973). The authors tested a large set of antibiotics, mostly with regard to the removal of bacterial contaminants. Less attention has been given to fungal contamination, though the fungal influence on the algal growth seems to be more expressive than that of bacteria.

The choice of an antibiotic should be considered from the point of view of its effect on eucaryotic cells. This, of course, limits the possibility to use

Tab. 1. — List of algal cultures and their contaminants

Algal culture	Contamination
Corcontochrysis noctivaga KALINA	bacteria
Ankistrodesmus spiralis (TURNER) LEMMERMANN	*Penicillium notatum* WESTL.
Coccomyxa simplex MAINX	*Aspergillus nidulans* (EIDAM) WINT.
Coelastrella striolata CHODAT var. *corcontica* HAVRÁNKOVÁ et KALINA	bacteria
Coelastrella striolata CHODAT	bacteria
Coelastrum sp.	*Tritirachium album* LIMBER + bacteria
Eremosphaera gigas (ARCHER) FOTT et KALINA	*Aspergillus versicolor* (VULL.) TIRABOSCHI + bacteria
Gloeocystis maxima MAINX	*Acremonium kiliense* GRŰTZ + bacteria
Chorella vulgaris BEIJERINCK var. *vulgaris*	*Penicillium* sp. + bacteria
Chlorococcum humicolum (NAEG.) RABENH.	*Acremonium kiliense* GRŰTZ
Chorococcum echinozygotum STARR	*Paecilomyces* sp. + bacteria
Chlorococcum vacuolatum STARR	*Acremonium kiliense* GRŰTZ
Chlorococcum wimmeri RABENHORST	*Paecilomyces* sp.
Mougeotia sp.	*Pacilomyces* sp. + bacteria
Oocystis solitaria WITTROCK f. *major* WILLE	*Penicillium notatum* WESTL. + bacteria
Pediastrum duplex MEYEN	*Paecilomyces* sp. + bacteria
Pteromonas sp.	*Paecilomyces* sp.
Scenedesmus brasiliensis BOHLIN	bacteria
Scenedesmus brasiliensis BOHLIN	*Actinomycetes* + bacteria
Scenedesmus denticulatus LAGERH.	bacteria
Scotiella sp.	*Verticillium lecanii* (ZIMM.) VIEGAS
Dunaliella acidophila (KALINA) MASJUK	*Cladosporium* sp.
Stigeoclonium sp.	*Actinomycetes* + bacteria
Tetraedron platyisthmum (ARCHER) G. S. WEST	*Acremonium* sp.

the large spectrum of antibiotics, and allows the application only of such preparations that are either harmless or do not cause genetical changes in algal cells.

MATERIALS AND METHODS

1. Cultures of algae and their contaminants

The contamination was followed in 24 cultures selected at random from the culture collection of the Department of Botany, Faculty of Science, Charles University, Prague. This number of species represents approximately 8% of the total collection. A list of the cultures and their contaminants will be found in Tab. 1. It is apparent from the table that the cultures of green algae (orders *Volvocales*, *Chlorococcales*, *Ulotrichales*, *Zygnematales*) were studied. Only *Corcontochrysis noctivaga* belongs to the class *Haptophyceae*. A survey of fungal contamination from the point of view of taxonomy and ecology has been published (BEDNÁŘOVÁ et FASSATIOVÁ 1976, in press).

2. Media

For cultivation of algae the medium ERB (KALINA 1975) was used. For its modifications the following abbrevitions are used:

ERB PG 1 contained: 0.05 g/l peptone and 0.05 g/l glucose.

ERB PG 2 contained: 0.5 g/l peptone and 0.5 g/l glucose.

Fungi were cultivated on Sabourad's glucose agar, Czapek-Dox agar and oat-meal agar. The medium for diffusion plate test was that described by ŘEHÁČEK (1958).

3. Antibiotics and their brief characteristics

Actidione. An antifungal antibiotic produced by strains of *Streptomyces griseus* (FORD et LEACH 1948). Characteristic of this antibiotic is the broad spectrum against fungi and no effect

against bacteria. Mode of action is the inhibition of DNA synthesis and protein synthesis (GOTTLIEB et SHAW 1970). PALMER et MALONEY (1955) found that blue-green algae are resistant to high concentrations of actidione, the growth of green algae and diatoms being inhibited by concentration of 50 μg/ml and lower. The cell division of *Haematococcus lacustris* is influenced by concentration of 0.016 – 0.032 μg/ml.

Preparation used: Actidione Upjohn, cryst.; soluble in water and alcohol.

Nystatin. An antifungal antibiotic produced by *Streptomyces aureus* (RAUBITSCHEK et al. 1952). The antibiotic is active against many fungi but no significant influence on bacterial cells was observed (BROWN et HAZEN 1957). Nystatin as a polyene antibiotic affects membrane permeability (GOTTLIEB et SHAW 1970). Blue-green algae are resistant up to the concentration of 200 μg/ml, green algae are sensitive to the concentration of 2 – 50 μg/ml (HUNTER et MCVEIGH 1961). LAMPEN et ARNOW (1961) observed similar sensitivity in *Euglenophyceae*, *Chrysophyceae* and *Xanthophyceae*.

Preparation used: Mycostatin Squibb, powder; soluble in water and ethanol.

Pimaricin (natamycin). A polyene antibiotic produced by *Streptomyces natalensis* (STRUYK et al. 1958). EBRINGER (1972) found that the lethal concentration for *Euglena gracilis* is 25 μg/ml

Preparation used: Pimafucin Mycofarm Delft, 2,5% sterile water suspension or the ointment (200 μg of pimaricin/g.) The antibiotic is separated from the ointment by its dilution in mixture ethanol-ether 3 : 1. Pimaricin is not soluble in water or ethanol.

Mucidin. A new antifungal antibiotic produced by the basidiomycete *Oudemansiella mucida* (MUSÍLEK 1965, MUSÍLEK et al. 1969). In comparison with nystatin and pimaricin, mucidin proved to be more active against 19 fungal species tested (ŠAŠEK et MUSÍLEK 1974a). In filamentous fungi and yeasts, mucidin induced morphological changes (ŠAŠEK et MUSÍLEK 1974b, c). ŠNEJDAR et al. (1973) describe the alterations in ultrastructure of the yeast *Candida pseudotropicalis*. The effect of mucidin on algae was not followed.

Preparation used: Mucidin cryst. 98%; Research Institute of Antibiotics and Biotransformations, Roztoky (batch 270169). Soluble in ethanol.

Ampicillin. An antibacterial antibiotic which inhibits the synthesis of mucopeptides in the cell wall. Compared to penicillin, ampicillin (aminobenzylpenicillin) shows broader spectrum of activity. The effect is on gram-positive as well as gram-negative bacteria.

Preparation used: Penbritin cryst.; Beecham Research Laboratories; soluble in water and ethanol.

4. Estimation of activity of antibiotics on isolated fungal contaminants

Antibiotic sensitivity of individual fungal species was tested by diffusion plate method as modified by ŠAŠEK and MUSÍLEK (1974c). The fungus was cultivated on SABOURAUD agar slants for 7 days. The mycelium with spores was dispersed in 5 ml of 0.1% Tween 80 and 2 ml of the suspension was used for surface medium inoculation. The solution or the suspension of the antibiotic in ethanol was applied into the wells in the agar plate in amount of 1 μg and 10 μg per well. The effectiveness of the antibiotic was estimated by the diameter of inhibition zones in mm.

5. The influence of pimaricin and mucidin on the algal cell morphology

The algae were cultivated on ERB medium in test tubes in daylight at room temperature. After several days of cultivation the ethanolic solution of antibiotic was added, its final concentration in medium being 2 μg/ml and 10 μg/ml, respectively. Control cultures were grown on medium with 1% ethanol. The samples were taken in regular intervals and examined microscopically. Photographs were made using a NfpK (Zeiss, Jena) microscope and an automatic FCM (Meopta) camera.

6. The influence of pimaricin and mucidin on the growth of algae

Algal suspension (1 ml) was inoculated into a test tube with 9 ml of ERB medium. After several days of cultivation the ethanolic suspension of the antibiotic was added into 10 test tubes, the final concentration in medium being 2 μg and 10 μg/ml, respectively. The second set of 10 test tubes — the control one — contained the ERB medium with 1% of ethanol. The growth was estimated as a change of extinction in 680 nm measured by a Specol spectrophotometer with extinction attachment for test tubes ER 1. The difference of growth curves was calculated by MUDRA's (1952) modification of t-zest.

7. Estimation of the effect of pimaricin and mucidin on fungal spores concentration in algal cultures

The contaminated algal culture was inoculated into an Erlenmeyer flask with ERB PG 1 medium. Ethanolic suspension (0.1 ml) of antibiotic was added to the resulting volume of 9.9 ml, the final concentration in medium being 10 μg/ml. In one set of experiments another dose of antibiotics was added after 12 days of cultivation. The cultures were grown in daylight at room temperature. The changes of spore concentration were estimated by spreading of 0.2 ml of culture on medium ERB PG 2 in Petri-dish. The samples were taken after certain intervals from each culture in 3 parallels. The number of spores was counted according to the number of colonies grown up within 48 hours.

RESULTS

1. The effectiveness of antibiotics on the isolated fungal contaminants

The influence of nystatin, actidione, mucidin and pimaricin was estimated by the diffusion plate method in eight fungal species isolated from algal cultures. The results are summarized in Tab. 2. Actidione, nystatin and

Tab. 2. — Effect of antibiotics on the isolated fungal species. The numbers indicate the diameter of inhibition zones (in mm) after 48 hours; indistinct zones in parentheses.

Fungi	Mucidin		Nystatin		Pimaricin		Actidione	
	1 μg	10 μg	1 μg	10 μg	1 μg	10 μg	1 μg	10 μg
Penicillium notatum	52	64	0	20	0	25	0	16
Paecilomyces sp.	16	18	0	20	0	25	0	0
	(30)	(42)	(17)		(15)		(25)	(37)
Acremonium sp.	0	16	0	16	0	27	0	20
	(30)	(38)					(18)	(28)
Aspergillus versicolor	0	0	0		0		0	0
				(30)		(29)		
Aspergillus nidulans	25	39	0	16	0	75	0	
								(15)
Acremonium kiliense	14	28	0	17	0	28	0	
								(15)
Penicillium sp.	34	47	0	21	0	30	0	
								(24)
Verticillium lecanii	19	28	0	17	0	29	0	0

pimaricin in the dose of 1 μg are ineffective, whilst the same dose of mucidin inhibits growth of seven fungal species; only *Aspergillus versicolor* is not inhibited by mucidin.

The dose of 10 μg of mucidin inhibits the growth of all fungal species tested except for *Aspergillus versicolor*. Pimaricin gives similar results, the inhibition zones against *Aspergillus versicolor* being inexpressive. In total, the inhibition zones induced by mucidin were larger than that of pimaricin; nystatin and actidione were less active than pimaricin. In view of these results, mucidin and pimaricin were chosen for the following experiment.

2. The effect of pimaricin and mucidin on the morphology of algal cells

Observations on the morphology of the algal cells were made simultaneously with the estimation of the growth curves.

Tab. 3. — Effect of antibiotics on the cell size of *Coelastrella striolata* CHODAT var. *corcontica* HAVRÁNKOVÁ et KALINA. — The table shows the relation between autospores or young cells (size 5—7 μm) and mature vegetative cells (size 7—12 μm); the abnormal cells grow up from 12.1—19.0 μm.

Antibiotic treatment (days)	Cells size (μm)	Percentage of the cells of the given size in culture		
		Control	Pimaricin 10 μm/ml	Mucidin 10 μ/ml
3	5.0—7.0	50.0	45.0	16.4
	7.1—12.0	50.0	55.0	77.9
	12.1—19.0	0	0	5.7
14	5.0—7.0	51.5	51.5	15.1
	7.1—12.0	48.5	48.5	79.2
	12.1—19.0	0	0	5.7
27	5.0—7.0	36.0	43.9	34.8
	7.1—12.0	63.0	56.1	58.4
	12.1—19.0	1.0	0	7.3

The following species of chlorococcal algae were studied:
Tetraedron caudatum (CORDA) HANSG., strain RŮŽIČKA 1962/18
Chlorococcum humicolum (NAEG.) RABENH.
Scotiella sp., strain KALINA 1966/1
Coelastrella striolata CHODAT var. *corcontica* HAVRÁNKOVÁ et KALINA, strain KALINA 1967/9; (a paper on this variety is now under preparation).

Morphological changes caused by pimaricin (shrinking of the protoplast and disintegration of the chloroplast) were observed only in *Chlorococcum humicolum*. On the contrary, in *Coelastrella striolata* var. *corcontica* a concentration of 10 μg/ml of pimaricin induced no morphological changes even after 27 days of cultivation.

Mucidin in concentration of 2 μg/ml brings about some morphological changes but its effect is more evident when concentration of 10 μg/ml is used. With this concentration the decrease of young cells in population after three days of cultivation is evident (Tab. 3). Some morphological changes are shown on Plate XI. Generally, mucidin induces separation of the protoplast from the cell wall, vacualization and cell rupture. Different light refracting bodies appear in the cells, some of them relatively big in size. They are mostly deposited between the cell wall and protoplast. After 27 days of cultivation 47% of dead cells were observed.

After 26 days of incubation in the presence of antibiotic 0.2 ml of the culture was spread on the ERB agar. After 20 days of cultivation, whole surfaces of all dishes were covered by the growth. Less extensive growth was observed in cultures treated by mucidin. No morphological changes were observed in these cultures.

3. The effect of antibiotics on algal growth

The growth curves of algae which are incubated in the presence of mucidin or pimaricin are shown in Figs. 1 and 2. The individual values represent the ratio of 10 extinction measurements at 680 nm.

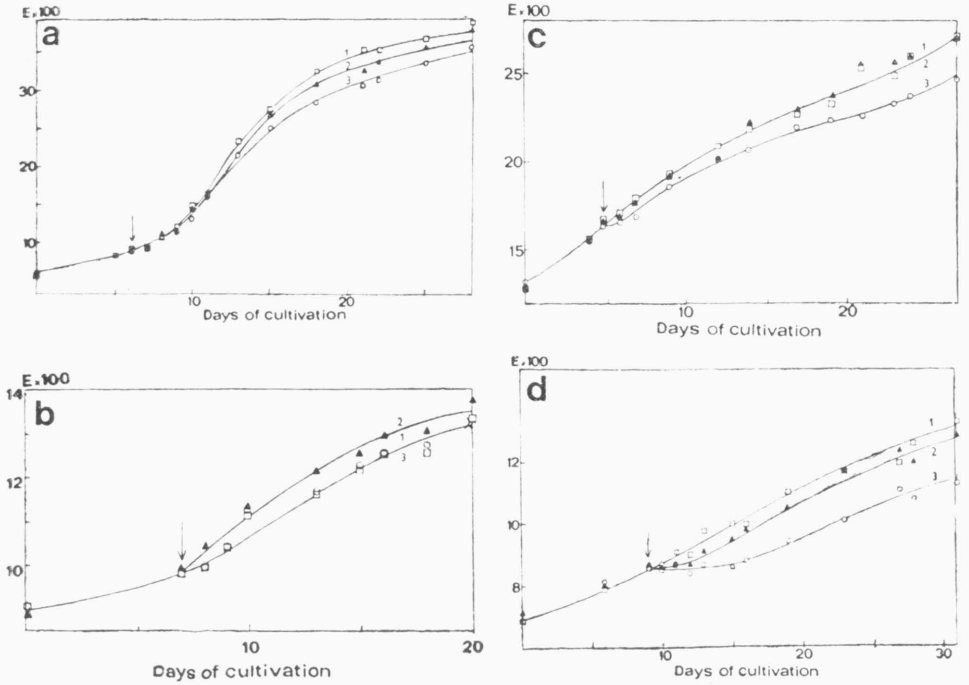

Fig. 1. — The effect of pimaricin on the growth of chlorococcal algae. – a: *Tetraedron caudatum* (Corda) Hansg. b: *Coelastrella striolata* Chodat var. *corcontica* Havránková et Kalina. c: *Scotiella* sp., strain Kalina 1966/1. d: *Chlorococcum humicolum* (Naeg.) Rabenh. — 1 — control culture with 1% of ethanol in medium; 2 — culture with pimaricin (2 μg/ml); 3 — culture with pimaricin (10 μg/ml). The arrow indicates the time of antibiotic addition.

Pimaricin (Fig. 1) in concentration of 2 μg/ml influenced the growth only very slightly. The only evidence of extinction decrease was registered in *Chlorococcum humicolum*. On the contrary, the growth rate of *Coelastrella striolata* var. *corcontica* was slightly higher in the presence of the antibiotic. Pimaricin in concentration of 10 μg/ml decreased slightly the growth of algae. A seven days' growth stagnation was estimated in *Chlorococcum humicolum*.

Mucidin (Fig. 2) in concentration of 2 μg/ml also influenced the growth very slightly. Shortly after the addition of the antibiotic, a growth stagnation lasting 2—3 days was registered. Mucidin in concentration of 10 μg/ml caused a growth retardation which was the most expressive in *Coelastrella striolata* var. *corcontica* and in *Tetraedron caudatum*.

A study of the influence of both antibiotics on some chlorococcal algae demonstrates that mucidin, especially in concentration of 10 μg/ml, induces the most pronounced changes in the morphology as well as in the growth rate of algal cells. The effect of pimaricin was less expressive. With regard to the algal species the effect of both antibiotics was different. For instance, in *Chlorococcum humicolum* the effect of pimaricin was distinct, whilst mucidin was not effective.

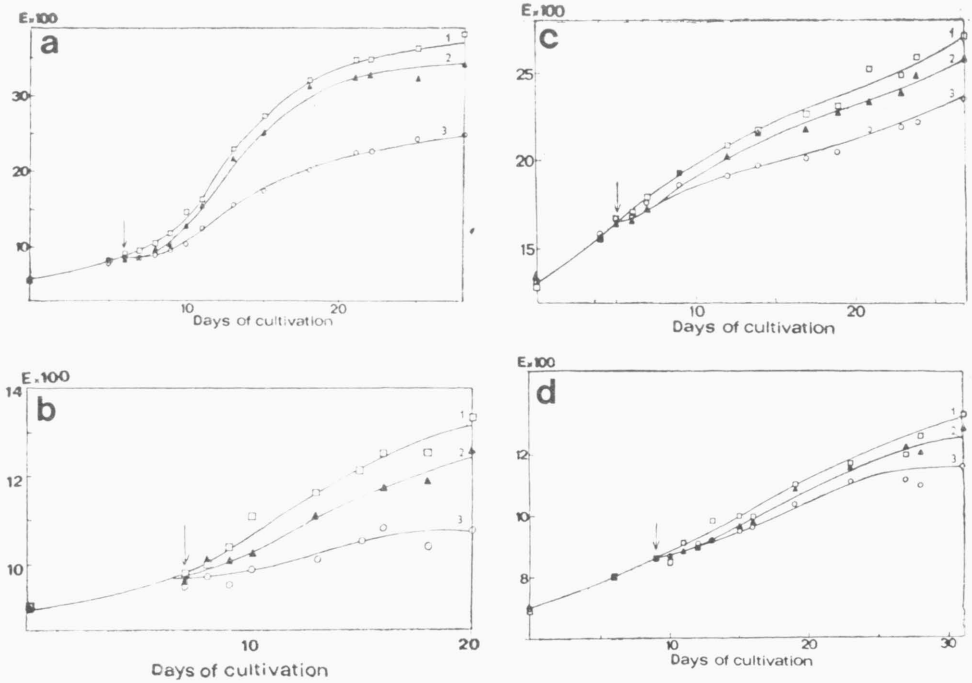

Fig. 2. — The effect of mucidin on the growth of some chlorococcal algae. — a: *Tetraedron caudatum* (CORDA) HANSG. b: *Coelastrella striolata* CHODAT var. *corcontica* HAVRÁNKOVÁ et KALINA. c: *Scotiella* sp., strain KALINA 1966/1. d: *Chlorococcum humicolum* (NAEG.) RABENH. — 1 — control culture with 1% of ethanol in medium; 2 — culture with mucidin (2 μg/ml); 3 — culture with mucidin (10 μg/ml).

4. The effect of pimaricin and mucidin on the concentration of fungal spores in the algal culture

Results of this study are summarized in Fig. 3. Each point of the curves which determines the number of fungal spores in 1 ml of the algal suspension, represents the average of five parallels. The full points indicate the simultaneous presence of bacteria.

The various experiments show the relationships among algae, fungi and bacteria after antibiotics application. The direction of the curves indicates the fungal spore concentration as well as the most suitable time for pure algal culture isolation.

For the inhibition of the fungus *Acremonium kiliense* in the culture of *Chlorococcum humicolum*, the antibiotic mucidin in concentration of 10 μg/ml was used (Fig. 3a). The highest decrease of fungal spores was observed after 12 hours of incubation and the low spores concentration increased but the growth of bacteria simultaneously decreased.

Cladosporium sp. causing the infection in *Scotiella* sp. (Fig. 3b) is very sensitive to mucidin (concentration 10 μg/ml). Maximal decrease of spore concentration appeared after 6 hours of incubation and the low spore concentration persisted till the end of the experiment. When pimaricin was applied the spore concentration decreased gradually and the minimum was re-

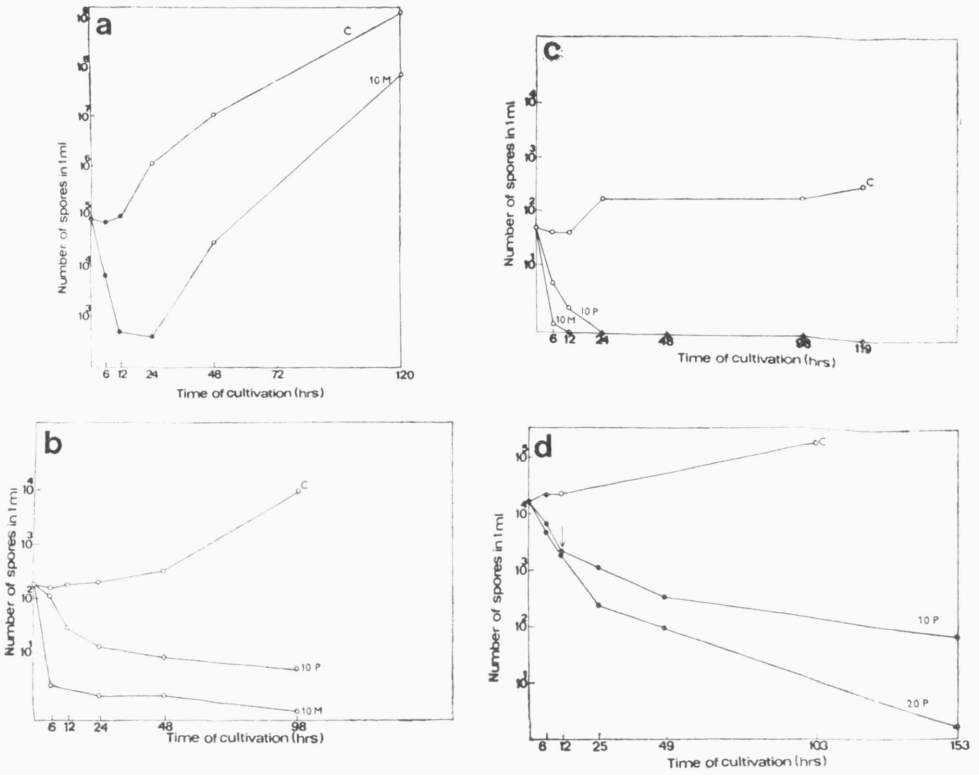

Fig. 3. — The effect of antibiotics on the fungal spores concentration in algal cultures. — a: *Acremonium kiliense* GRŮTZ in culture of *Chlorococcum humicolum* (NAEG.) RABENH. b: *Cladosporium* sp. in culture of *Scotiella* sp., strain HOLUBCOVÁ 63/1. c: *Chaetomium* sp. in culture of *Scotiella* sp., strain HOLUBCOVÁ 63/1. d: *Penicillium notatum* HESTL. in culture of *Oocystis solitaria* WITTR. f. *major* WILLE; second dose of pimaricin added (arrow). — C — control; 10 P — 10 μg/ml of pimaricin; 20 P — 20 μg/ml of pimaricin; 10 M — 10 μg/ml of mucidin; 20 M — 20 μg/ml of mucidin. The dots indicate the simultaneous presence of bacteria.

ached after 98 hours of cultivation. No bacteria were observed throughout the experiment.

Chaetomium sp. in the culture of *Scotiella* sp. (Fig. 3c) showed almost the same sensitivity to pimaricin and to mucidin (concentrations of 10 μg/ml). With mucidin, the minimum concentration of spores was after 6—12 hours of incubation, with pimaricin after 12—24 hours. No fungal spores appeared in prolonged cultivation. No bacteria were observed throughout experiments.

Penicillium notatum in the culture of *Oocystis solitaria* WITTR. f. *major* WILLIE (Fig. 4) is less sensitive to mucidin in concentration of 10 μg/ml. The minimal spore concentration was after 6 hours of incubation, after which the spore number increased. In Fig. 4, curve 20 M indicates the results of another dose of mucidin after 12 hours; the decrease of spore concentration is evident. Bacteria were observed only when fungal spore concentration was minimal.

Fig. 3d shows the effect of pimaricin on *Penicillium notatum* in culture of *Oocystis solitaria*. Pimaricin brings about slow spore decrease; minimal

concentration was after 50 hours of cultivation. A new dose of pimaricin (10 μg/ml) after 12 hours caused further spore concentration decrease.

The experiments described indicate that the effect of the antibiotics varies from one fungal species to another. Generally, the effect of mucidin is rapid but the spore decrease is short-term, whilst the effect of pimaricin is rather slow but long-lasting. The addition of a new dose of the antibiotic (e.g. after

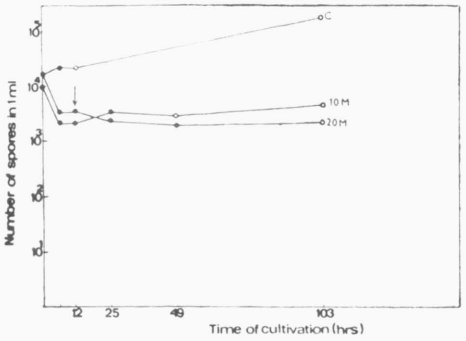

Fig. 4. — The effect of mucidin on the concentration of spores of *Penicillium notatum* WESTL. in the culture of *Oocystis solitaria* WITTR. f. *major* WILLE; second dose of mucidin added (arrow). — C — control; 10 M — 10 μg/ml of mucidin; 20 M — 20 μg/ml of mucidin. — The dots indicate the presence of bacteria.

12 hours) can be advantageous, resulting in an additional fungal spore decrease. However, tolerance of the algal culture to this antibiotic concentration is important.

Mucidin as well as pimaricin cause only rarely a total disappearance of fungal spores. Some spores still remain in the algal population. The minimal spore concentration is the most suitable period for the isolation of a pure algal culture.

An interesting correlation between bacteria and fungi was observed. Bacterial infection increased rapidly when the fungal spore concentration was minimal. It is not clear whether this is a nutritional competition or an inhibition by fungal metabolites. A simultaneous removal of fungal as well as bacterial contamination is important from the point of view of the algal cultures purification.

5. Purification of contaminated algal cultures

The first step is the rough estimation of the contaminant. For the removal of bacteria, the use of ampicillin in concentration of 1000 μg/ml is recommended. When fungal infection is also present, the mixture of ampicillin (1000 μg/ml) and pimaricin 10 μg/ml) is suitable. Antibiotics are freshly prepared as water solution and suspension, respectively.

The following procedure for the algal collection purification is proposed:

a) Into Erlenmeyer flask (25—50 ml), 8 ml of sterile ERB PG 1 solution, 1 ml water solution of ampicillin (10 000 μg/ml), 0.1 ml of pimaricin (100 μg/ml) and 1 ml of algal cells suspension (density 3000—5000 cells/ml) is added. The culture is incubated under light at room temperature.

b) After 24, 48, 72 (exceptionally 96) hours, 0.2 ml of algal suspension is

spread over agar medium ERB PG 2 in Petri dish. All work is done aseptically in Hansen chamber with UV light.

c) The cultures on dishes are exposed to day-light or artificial light. During the checks after 12, 24 and 48 hours the following situations usually occur:

1) Inoculation (24 hours of antibiotic treatment) — dense growth of contaminants all over the dish.
2) Inoculation (48 hours of antibiotic treatment) — mostly dense cover of contaminants but sometimes it is possible to pick up the pure algal colony.
3) Inoculation (48 hours of antibiotic treatment) — usually the most suitable for algae isolation; colonies of fungi are rare, bacteria are absent.
4) Inoculation is not usually necessary and, moreover, mostly not practicable because of progressing fungal spore concentration.

d) The pure algal colonies are picked up under preparative microscope in Hansen chamber. Using a platinum needle, the pure colonies are transferred in ERB PG 2 agar medium in the Petri dish. The visual control of purity is done after 2, 3 and 4 days of growth.

e) The pure algal cultures are transferred into test tubes with ERB agar.

After some training and skill, the repetition of the procedure is not reasonable for the same culture. The possible failure is due to the low sensitivity of the fungus to the antibiotic or high sensitivity of the algae.

DISCUSSION AND CONCLUSIONS

The algal cultures of the collection of the Department of Botany proved to contain fungal contaminants and bacteria. It is interesting to note that in each individual algal culture mostly one fungal species was detected. An antagonistic relationship between bacterial and fungal contamination was observed.

The contamination could be of different origin. Bednářová et Fassatiová (1976, in the press) report that some fungi accompany the algae from the original source of isolation, the others get into the algal culture in the course of manipulation in laboratory.

The relation between contaminants and algae in culture depends on biological activity of both. Even though it seems that bacteria in algal cultures are less harmful than fungi, Schnepf et al. (1974) proved that some bacteria invade the algal cells. In the culture we worked with, the bacterial contamination was completely removed by means of ampicillin. Successful results were obtained also in algal cultures producing much of slime (*Corcontochrysis*).

We did not follow the effect of ampicillin on algal cell. However, the experiment with the removal of the contamination (Fig. 4) indicates that algae tolerate ampicillin in concentration of 1000 μg/ml. The treatment of the cultures of *Coelastrella costata, Coelastrella striolata* var. *corcontica* by ampicillin in concentration 2000—6000 μg/ml showed no effect on the growth rate.

The problem is, how to remove this contamination reliably in a short time. We have used many recommended procedures but to no avail. The mechanical purification is suitable for slightly contaminated cultures. Generally, growth of algae is much slower than that of fungi. The differences in growth rate support the expansion of fungal contaminants in algal culture which complicates the culture purification.

Tab. 4. — Purification of algal cultures

Algal culture (listed according to PUNČOCHÁŘOVÁ et al., 1975)		Contaminant		Antibiotics		Post-treatment contamination			Axenic culture obtained
		Bacteria	Fungi	Ampicillin µg/ml	Pimaricin µg/ml	Bacteria	Fungi	Bleaching effect	
Botrydiopsis intercedens	D 301	+	+	1 000	10	+	+	+	–
Bumilleriopsis filiformis	D 101	+	+	1 000	10	–	–	–	+
Carteria lunzensis	G 903	+	+	1 000	5–10	–	–	–	+
Chlamydomonas eugametos	G 221	+	+	1 000	10	–	+	+	–
Chlamydomonas hydra	G 215	+	+	1 000	5–10	–	–	–	+
Chlamydomonas eugametos	G 218	+	+	1 000	5–10	–	+	+	–
Chlorella fusca	H 1967	+	+	1 000	10	–	–	–	+
Chlorella minutissima	H 1924	+	+	1 000	10	–	+	+	+
Chlorella vulgaris	H 1955	+	+	1 000	10	–	–	+	+
Chlorsarcinopsis aggregata	J 701	+	+	1 000	10	–	–	–	+
Coelastrella stiolata var. *striolata*	H 3602	+	+	2 000	10	–	–	–	+
Coelastrella striolata var. *corcontica*	H 3603	+	–	1 000	–	–	–	–	+
Coelastrella costata	H 3601	+	+	4 000	10	–	–	–	+
Corcontochrysis noctivaga	D 101	+	–	1 000	–	–	–	–	+
Dunaliella acidophila	G 301	+	+	1 000	10	+	+	–	–
Enalax alpinus	H 4701	+	+	1 000	10	–	–	–	+
Gloeococcus maximus	G 601	+	+	1 000	10	–	–	+	–
Heterothrix solida	D 201	+	+	1 000	10	–	–	–	–
Neochloris aquatica	H 2701	+	–	1–2 000	10	–	–	+	–
Scenedesmus costatus	H 504	+	–	1 000	–	–	–	–	+
Scotiella oocystiformis	H 4401	+	–	1 000	10	–	–	–	+
Spongiochloris excentrica	H 2602	+	+	1 000	20	–	–	–	+
Spongiochloris spongiosa	H 2601	+	+	1 000	10	–	–	–	+

The use of antibiotics seems to be one possible approach. Since many antifungal antibiotics are not produced commercially, we tested both recommended and available ones. Actidione and nystatin were shown to be unsuitable. However, in bluegreen algae which tolerate high concentrations of these antibiotics (PALMER et MALONEY 1955, HUNTER et MCVEIGH 1961), the situation could be different. Pimaricin and mucidin are more convenient for algal culture purification but information about their effect on the algal cells is scarce.

Pimaricin belongs to the polyenic antibiotics which in eucaryotic cells induce changes in permeability and damage of cell membranes. The sterols seem to be the specific sites in the membranes that are alterated or attached by the polyens. In this respect the binding activity of pimaricin is lower than that of nystatin (RAAB 1972). The algal cells are affected by pimaricin in different degrees. PERLMAN (1965) reported the inhibition of growth in *Scenedesmus* by pimaricin in concentration of 10 µg/ml, whilst in *Chlorella* the inhibition dose was 1 µg/ml (EBRINGER 1972). The antibiotic action can be attenuated by the composition of the nutrient medium (LAMPEN

et al. 1962). It is also necessary to mention the observation of WHIFFEN (1948) and ZEHNDER et HUGHES (1958) that certain concentration of an antibiotic affects the algal cells according to its suspension-density.

Pimaricin in concentration of 10 μg/ml seems to be less harmless for the algal cells than mucidin. Pimaricin caused slow but long-term decrease of fungal spore concentration.

Mucidin is a new antifungal antibiotic and was tested in this algal culture for the first time. Mucidin caused growth inhibition as well as morphological changes in algal cells. Similar morphological changes were observed by ŠAŠEK et MUSÍLEK (1974b) in the yeast *Candida pseudotropicalis*, namely in the shape and size of the cells. The cells treated by mucidin were larger and almost sphaerical. We observed the same phenomenon in morphology of *Coelastrella striolata* var. *corcontica*. Other species (*Chlorella vulgaris*, *Carteria lunzensis*, *Chlorococcum* sp.) were not affected by mucidin in concentration 10 μg/ml.

The results obtained by the method described above are summarized in Tab. 4. From 23 algal cultures purified, 16 pure cultures resulted; in 7 cultures the method was not successful even though the procedure was repeated and the antibiotic concentration changed. Different sensitivity to pimaricin of different algal species (see Tab. 4) is not in correlation with the taxonomic position or the structure of the thallus.

In several species we observed the bleaching effect. In *Botrydiopsis intercedens*, *Chlamydomonas eugametos*, *Chlorella minutissima*, *Gloeococcus maximus* and *Neochloris aquatica*, the cell bleaching started either in the time of the first contact with the antibiotic or after the transfer into the normal medium. In contrast to the results of EBRINGER (1972) in our experiments the bleached cells stopped their reproduction and perished.

The axenic cultures obtained by the method described do not show any morphological changes. If in some cases a small number of bleached cells appeared, they faded away gradually. The described method of the axenisation of algal cultures is advantageous for many algal species. It is specially useful when other methods of purification fail.

SOUHRN

Autoři zjišťovali možnosti odstranění houbových kontaminantů řasových kultur pomocí nystatinu, aktidionu, pimaricinu a mucidinu. Účinky těchto antibiotik sledovali u osmi druhů hub, izolovaných z řasových kultur. Nejširší spektrum působení a největší účinek vykazoval mucidin a pimaricin. Pokusně se podařilo najít vhodnou koncentraci, neškodnou pro růst řas, ale omezující růst hub. Mucidin působí již v koncentraci 2 μg/ml, pimaricin v koncentraci 10 μg/ml. Potlačení houbové kontaminace mělo často za následek rychlé přemožení bakteriálních kontaminantů. Bakterie se podařilo odstranit pomocí ampicillinu v koncentraci 1000 μg/ml. V závěru práce je doporučena rutinní metoda čistění řasových kultur.

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See also Plate XI. in the Appendix.

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Rodák ze Zvole nad Pernštejnem. Zprvu asistent Hospodářské akademie v Táboře, později profesor a ředitel středních zemědělských škol v Hořicích v Podkrkonoší, v Kuklenách a v Kadani, šlechtitel a lichenolog. Jako absolvent přírodních věd na filosofické fakultě UK se věnoval lichenologickému výzkumu převážně na území Československa a Balkánu, ale zpracoval bohatý herbářový materiál rovněž ze záp. Evropy i odjinud. Dlouholetá specializace na nižší, pyrenokarpní lišejníky jej přivedla k sepsání monografického díla „Československé lišejníky čeledi *Verrucariaceae*" (1954) a k spoluautorství na „Klíči k určování lišejníků ČSR I" (1956). Kromě toho publikoval několik desítek původních lichenologických prací (zvláště o čeledích *Verrucariaceae* a *Dermatocarpaceae*) i článků šlechtitelských a didaktických. Jeho lichenologický herbář je z největší části v Národním muzeu v Praze.

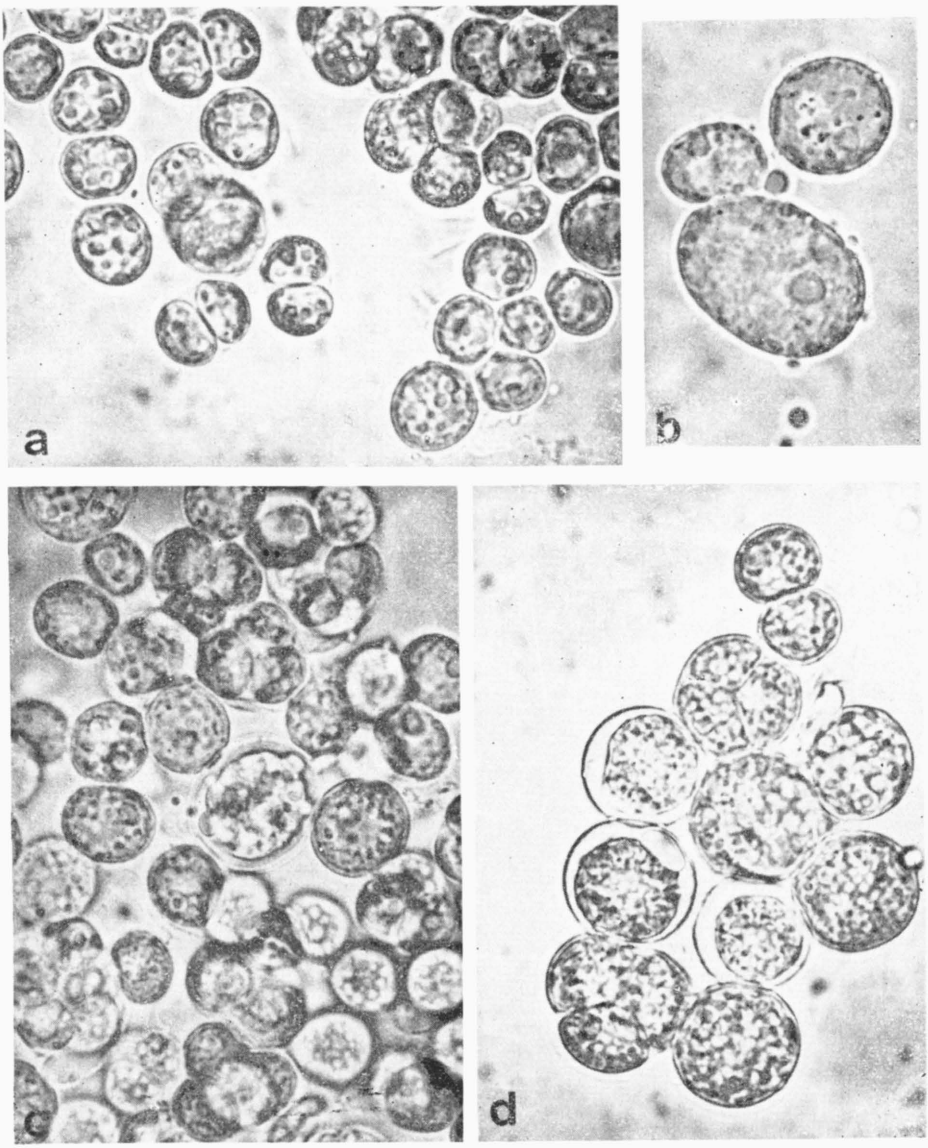

Plate XI. — *Coelastrella striolata* CHODAT var. *corcontica* HAVRÁNKOVÁ et KALINA. The effect of mucidin in concentration of 10 μg/ml on cell morphology: a, control culture after 14 days of cultivation, 1560×; b, culture treated by mucidin for 3 days, 1920×; c, culture treated by mucidin for 6 days, 1560×; d, culture treated by mucidin for 14 days, 1560×.