ACTA
MYCOLOGICA
vol. VIII, 1–1972

Nutrient solution requirements of representatives of the *Penicillium clavigerum* section

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It appeared that the species from the *Penicillium clavigerum* section require for optimum growth a nutrient solution composed of: 0.2 g NaCl; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.1 g $CaCl_2$; 0.87 g KH_2PO_4; 0.14 g K_2HPO_4; 4.05 mg $ZnSO_4 \cdot 7H_2O$; 4.43 mg $MnSO_4 \cdot 4H_2O$; 0.5 mg $FeSO_4 \cdot 4H_2O$, the presence of vitamins from the group B and of organic N being essential.

INTRODUCTION

The section of *Penicillum clavigerum* is represented by three species. One of them, namely *P. clavigerum*, thrives on simple synthetic media, while the two other species, *P. claviforme* and *P. isariaeforme*, require a medium supplemented by yeast water (Piskorz 1967). It is known that yeast water is rich in nitrogen and contains vitamins; the composition is not exactly known, and presumably varies depending on the baker yeasts used. In some physiological investigations it is necessary to know the exact composition of the medium on white the organisms dealt with are grown, and, therefore, the aim of the present study was to find a synthetic medium warranting the optimum growth and development of *P. claviforme* and *P. isariaeforme*.

MATERIAL AND METHODS

The experiments concerned stocks of *P. claviforme* and *P. isariaeforme* obtained from the Centraalbureau voor Schimmelcultures in Baarn, Holland. On the basis of results of previous studies (Piskorz 1967 a, b) the moulds were grown in thermostats in darkness and in light at the optimum intensity of 900 lx, at 22°C for 7 days (*P. claviforme*) and at 25°C for 12 days (*P. isariaeforme*).

The composition of the media used will be presented below. The media were liquid (20 ml of medium in a 100 ml Erlenmayer flask), excepted in additional cultures destined for morphological measurements and grown in Petri rishes on media solidified by agar. The agar discs method (P a s i u t 1970) was used in the study of the sources of carbon. The amount of nitrogen in the yeast water and in casein was determined at the Institute of Organic Chemistry of the Jagellonian University.

The criteria used for the evaluation of media were the dry weight of the mycelium, the size and shape of the coremia. In some cases, before inoculation and after completion of growth, the pH of the medium was determined.

INFLUENCE OF COMPOSITION OF BASAL MEDIUM ON GROWTH AND DEVELOPMENT OF *P. CLAVIFORME* AND *P. ISARIAEFORME*

The simple medium used in previous investigations contained 50 g glucose, 1 g NH_4NO_3, 1 g $MgSO_4 \cdot 7H_2O$, 1 g K_2HPO_4, 0.003 g $ZnSO_4 \cdot 7H_2O$ and 0.001 g $FeSO_4 \cdot 4H_2O$ dissolved in 1000 ml of yeast water. This medium allows perfect growth and undisturbed photomorphogenesis. Nevertheless, in some cases it is necessary to grow the microorganisms on a medium whose composition is exactly known. The search for such a medium began by attempts to modify the medium hitherto used. It soon appeared that the basal medium deprived of yeast water did not allow optimum growth of the moulds dealt with.

The well developed basal mycelium produced scarce and non typical coremia set in groups. It appeared also that the medium contained an optimum amount of glucose, as its decrease resulted in smaller crops of the mycelium. Analyses of yeast water indicated low amounts of reducing sugars and comparatively high amounts of nitrogen. Therefore, nitrogen (in the form of ammonium nitrate) was added up to the amount present in the basal medium with yeast water. The expected effects did not take place and, therefore, nitrogen in the form of amino acids was added instead of inorganic nitrogen. Casein was added to the medium in quantities containing amounts of nitrogen equal to that in 1 g of ammonium nitrate. However, it appeared that both species inadequately assimilate the complex of amino acids given in the hydrolisate of casein. Attempts to modify the basal medium were closed by a study of the action of various salts containing nitrogen in the form of nitrate or ammonium on the growth and development of *P. claviforme* and *P. isariaeforme*. The investigations concerned exclusively cultures grown

Table 1

Action of modification of the basal medium (50 g glucose, 1 g NH_4NO_3, 1 g $MgSO_4$, 1 g K_2HPO_4, 0.001 g $FeSO_4$, 0.003 g $ZnSO_4$ per 1000 ml of yeast water) on the growth of *P. claviforme* and *P. isariaeforme*, expressed in mg of dry weight

Modification of medium	*P. claviforme*		*P. isariaeforme*	
	Light	Darkness	Light	Darkness
Basal medium (BM)	240.0	273.0	270.0	188.0
Synthetic medium (MS) composition as BM without yeast water	145.0	122.7	120.0	80.0
Synthetic medium (MS) 3% glucose	107.6	86.6	124.0	53.0
Synthetic medium (MS) N as NH_4NO_3 in amount equal to that in BM	52.8	44.1	187.0	161.0
Synthetic medium (MS) amount of casein equivalent to the amount of N in 1 g NH_4NO_3	85.5	73.2	—	—
Synthetic medium (MS), amount of KNO_3 equivalent to the amount of N in 1 g NH_4NO_3	98.4	—	—	—
Synthetic medium (MS) amount of $Ca(NO_3)_2$ equivalent to the amount of N in 1 g NH_4NO_3	78.6	—	—	—
Synthetic medium (MS) amount of $(NH_4)_2SO_4$ equivalent to the amount of N in 1 g of NH_4NO_3	48.7		—	—

in light, as it appeared previously that inorganic nitrogen both as the ion NH_4^+ and NO_3^- — is probably poorly assimilated by both species. The results presented in table 1 fully agree with this supposition.

CULTURES OF *P. CLAVIFORME* AND *P. ISARIAEFORME* GROWN ON OTHER SYNTHETIC MEDIA

The experiments described above clearly indicate that *P. claviforme* and *P. isariaeforme* may be grown on the hitherto used medium only if prepared on yeast water. As yeast water, besides considerable amounts of nitrogen, contains vitamins, it was expected that addition of vitamins to the medium would stimulate the growth and development of the species dealt with. In preliminary investigations, a medium rich in different substances, used by Daniel and Baldwin (1964) to grow the plasmodia of *Myxomycetes*, was used. This medium contained — besides glucose

and a small amount of citric acid as a source of carbon and ammonium chloride as a source of nitrogen — macro- and microelements and the following vitamins: inositol, choline hydrochloride, biotin, thiamine hydrochloride, pyridoxal hydrochloride, pyridoxine, hydrochloride, niacin, calcium panthotenate, p-aminobenzoic acid, folic acid, vitamin B_{12}, riboflavin and also a mixture of amino acids: dl-methionine, glycine, arginine, hydrochloride, l-cysteine hydrochloride, l-histidine hydrochloride, l-leucine, l-lysine hydrochloride, dl-isoleucine, dl-phenylalanine, dl-tryptophan, dl-serine, dl-threonine, dl-valine; besides 0.2 ml of 0.05% solution of hematin was added to each flask. The action of the medium without

Table 2

Influence of various sources of C on growth and dry weight of the mycellium of *Penicillium claviforme* and *Penicillium isariaeforme* (Fries medium an addition of 1 mg thiamine, amount of asparagine equivalent to the amount of N in 1 g of ammonium nitrate)

Source of carbon	*Penicillium claviforme*		*Penicillium isariaeforme*	
	Light	Darkness	Light	Darkness
Control (medium with yeast water)	7.63	7.05	6.45	4.50
Glucose	6.94	5.85	2.02	1.80
Fructose	4.92	5.58	6.18	5.44
Ribose	4.62	3.96	2.44	2.34
Xylose	3.94	3.76	2.74	2.29
α-ketoglutaric acid	2.40	3.02	0.10	0.10
Galactose	5.48	7.17	3.59	3.36
Saccarose	8.19	6.25	6.61	4.10
Sodium succinate	0.10	0.10	0.08	0.05
Glycogen	1.05	1.31	0.05	0.05

hematin was studied, too. The results presented in table 2 indicate that the medium is not suitable for the culture of the microorganisms dealt with.

On the other hand, positive results were obtained using the medium prepared for cultures of species of the genus *Coprinus* (Fries 1955). This medium was modified, the concentration of glucose being not only 2%, but also 3% and 5%. In each experiment 3.330 g of asparagine, containing the amount of nitrogen equal to that in 1 g of ammonium nitrate, per 1 l of medium was added; the action of a higher concentration of asparagine, namely 5%, was also studied. The Fries medium contained, together with the substances mentioned above, 0.2 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 0.87 g KH_2PO_4, 0.14 g

K_2HPO_4, 4.05 mg $ZnSO_4 \cdot 7H_2O$, 4.43 mg $MnSO_4 \cdot 4H_2O$, 0.05 mg $FeSO_4 \cdot 4H_2O$, instead of iron citrate used by Fries. The data presented in Table II imply that this medium is suitable for cultures of *P. claviforme* and *P. isariaeforme*. This may be explained first and foremost by the form in which nitrogen was supplied, both species assimilating nitrogen best in the form of amide. *P. isariaeforme* assimilates equally well from a mixed source of nitrogen, namely asparagine and ammonium tartrate, while only asparagine is the best source for *P. claviforme*. Best results have been obtained with a 5% concentrations of glucose and asparagine. Growth on such a medium is equal to that observed on the medium containing yeast water, as evaluated by the dry weight of the mycelium.

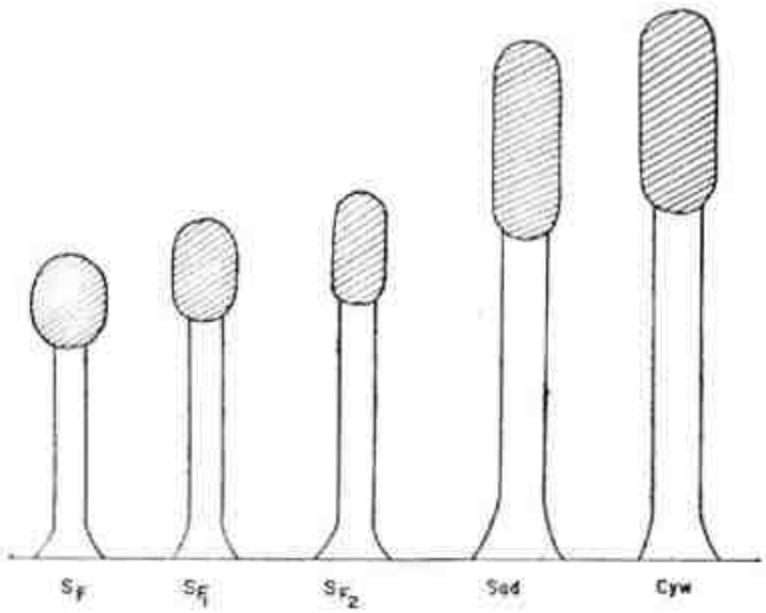

Fig. 1. Shape (schematic) of coremia of *P. claviforme* grown in light as depending on the concentration of thiamine added to the Fries medium. S_F — synthetic medium; S_{F_1} — same medium, 0.01 mg thiamine added; S_{F_2} — same medium 0.1 mg thiamine added; S_{ad} — medium with 1 mg thiamine and with asparagine; C_{yw} — control culture grown on medium with yeast water

When applying the modified Fries medium, the action was studied of the different concentrations of thiamine on the growth and development of the moulds dealt with. The following concentrations were used: 10 mg, 1 mg, $1 \cdot 10^{-1}$, $1 \cdot 10^{-2}$, $1 \cdot 10^{-3}$ mg of thiamine per 1 l of medium. It appeared that none of these influenced the dry weight of the mycelium in either species, but that the presence of thiamine improved the morphology of coremia. Therefore, 1 mg of thiamine was always added to the medium. (Fig. 1).

ACTION OF THE VARIOUS SOURCES OF CARBON ON THE GROWTH AND DEVELOPMENT OF *P. CLAVIFORME* AND *P. ISARIAEFORME*

Glucose was the source of carbon both in the Fries medium used in cultures of species belonging to the genus *Coprinus*, and in the modified Fries medium used in cultures of the moulds *P. claviforme* and *P. isariaeforme*. Thus it became necessary to decide whether there existed other sources of carbon better utilized by the species dealt with. The method of cellophane-agar discs 10 mm in diameter and 5 mm high was that used in our experiments. This allowed to use small quantities of various sources of carbon and to increase the number of repetitions. The growth curves of *P. claviforme* and *P. isariaeforme* grown in light and in darkness on the modified Fries medium were first established. The time of cultures was six days for *P. claviforme* and ten days for *P. isariaeforme*. (Figs. 2, 3). The results of the experiments, presented in table 3, suggest glucose, saccharose, fructose to be the best sources of carbon for *P. claviforme*. When grown on these sugars, the mould developed well, the amount of dry weight was high, coremia normal and sporulation abundant. For the other species *P. isariaeforme*, glucose is also

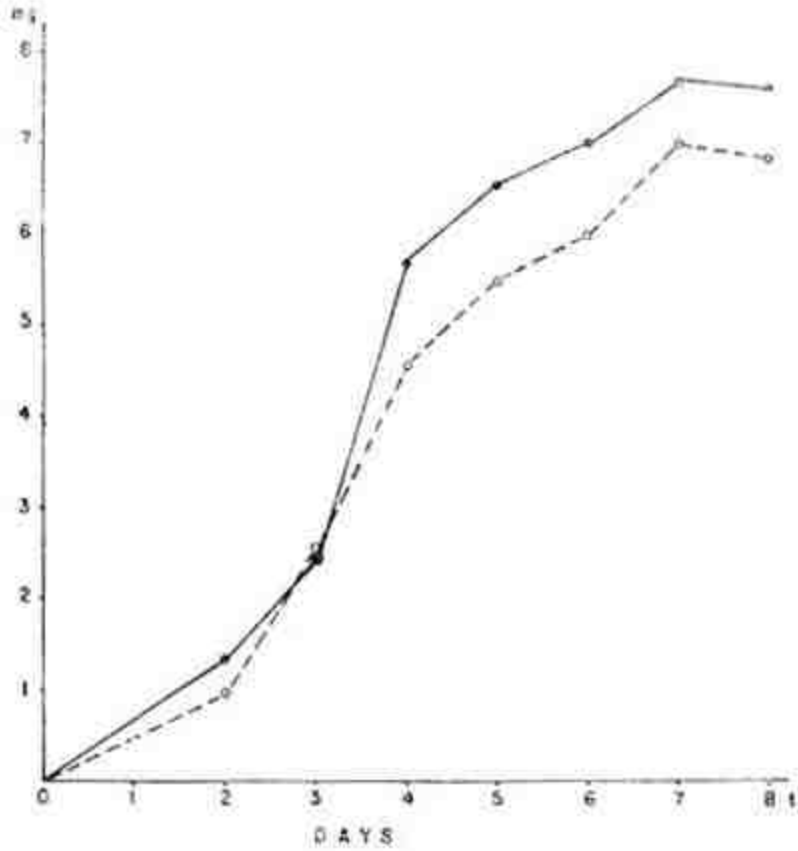

Fig. 2. Time of culture of *P. claviforme* grown on agar-cellophane discs consisting of Fries medium with asparagine added; black circles — cultures grown in light, white circles — cultures grown in darkness

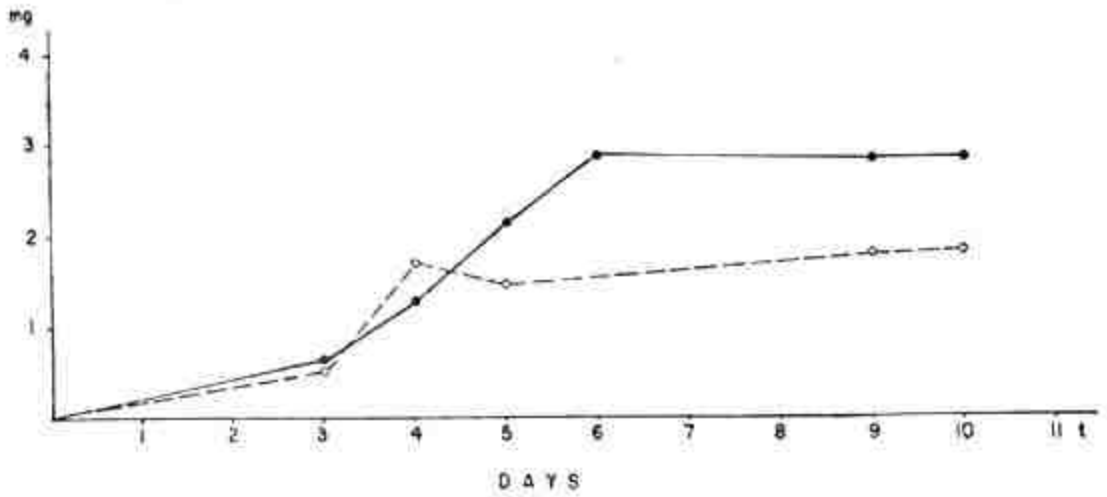

Fig. 3. Time of culture of *P. isariaeforme* grown on agar-cellophane discs consisting of Fries medium with asparagine added; black circles — cultures grown in light, white circles — cultures grown in darkness

Table 3

Cultures of *Penicillium claviforme* and *Penicillium isariaeforme* grown on various synthetic media

Medium	*Penicillium claviforme*		*Penicillium isariaeforme*	
	Light	Darkness	Light	Darkness
Medium B and D without hematin	13.0	13.0	24.8	25.6
Medium B and D without hematin	13.0	14.0	26.9	27.8
Synthetic medium (MS) 3% glucose	107.6	86.6	124.0	53.0
Synthetic medium (MS) 5% glucose	145.0	122.7	120.0	80.0
Synthetic medium Fries (MSF) 3,330 g asparagine, 2% glucose	79.1	79.5	—	—
Synthetic medium Fies (MSF) 3,330 g asparagine, 3% glucose	139.9	117.6	186.0	160.7
Synthetic medium Fries (MSF) 3,330 g asparagine, 5% glucose	159.0	133.0	211.5	196.5
Synthetic medium Fries (MSF) 5 g asparagine, 5% glucose	228.0	213.0	213.0	194.6
Synthetic medium Fries (MSF) 5 g asparagine, 1 g ammonium tartrate	158.7	146.0	259.9	241.9

Table 4

Comparison of development of *Penicillium claviforme* and *Penicillium isariaeforme* grown on optimum media

Medium	*Penicillium claviforme*		*Penicillium isariaeforme*	
	Light	Darkness	Light	Darkness
Synthetic medium Fries 12.5 g glucose, 12.5 g fructose, 1 mg vitamin B_1	244.5	264.3	273.8	248.0
Synthetic medium Fries, ammonium tartrate, 12.5 g glucose, 6.25 g fructose, 6.25 g saccharose, 1 mg vitamin B_1	—	—	259.9	249.1
Synthetic medium Fries, 12.5 g galactose, 12.5 g fructose, 1 mg vitamin B_1	274.6	251.0	—	—

a suitable source of carbon. The results shown in table 4 imply that for *P. isariaeforme* asparagine, supplied together with ammonium tartrate, adequately replaces the medium containing yeast water previously used.

EVALUATION OF RESULTS

P. claviforme and *P. isariaeforme* grown on a simple medium containing yeast water (Piskorz 1967, 1968) form a thick layer of mycelium with well developed coremia. The same medium devoid of yeast water is apparently unsuitable for either species, the growth and development being clearly stunted. Also in *Aspergillus giganteus*, if grown on a medium devoid of yeast water, the dry weight of the mycelium is reduced by half, though the development is not visible disturbed (Zurzycka 1955). These results seem to indicate that the microorganisms dealt with have been given unsuitable sources of nitrogen or of carbon, or that for normal growth they needed some substances from the group of vitamins or other growth factors. It is known (Cochrane 1958; Lilly and Barnett 1951) that fungi are able to assimilate nitrogen from nitrate and ammonium salts or from organic substances. Cantino (1955) is of the opinion that the ability to utilize nitrate implies

N-autotrophism in fungi, as this is just what the typical autotrophs do. *P. claviforme* and *P. isariaeforme* are able to utilize in a slight degree the nitrogen contained in nitrates; but nitrogen in the form of amide as asparagine suits them best. The results of the experiments do not indicate that amino acids are a good source of nitrogen for the microorganisms dealt with. *P. isariaeforme* assimilates well from mixed sources of nitrogen, composed of ammonium tartrate and asparagine, resembling in this respect some species of the genus *Coprinus* (F r i e s 1955). Moreover, both species dealt with here require similar media, which are also similar to those required by the species of *Coprinus*, as implied by the successful replacement of medium with yeast water by a slightly modified F r i e s (1956) medium.

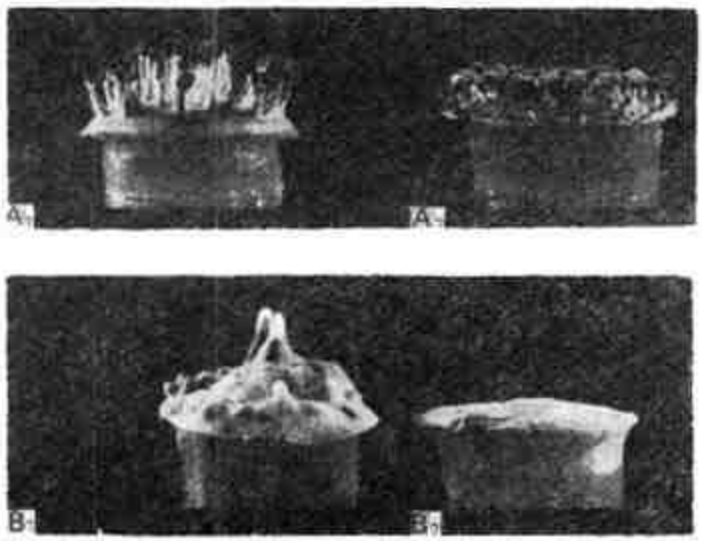

Fig. 4. Cultures obtained on optimum medium (Fries medium with 1 mg thiamine, 5 g asparagine and a mixed source of carbon) A_1 — *P. claviforme* grown in light, A_2 — grown in darkness, B_1 — *P. isariaeforme* grown in light, B_2 — same species grown in darkness

The species dealt with here assimilate carbon best from the following sugars: glucose, fructose and galactose (*P. claviforme*) and glucose, saccharose and fructose (*P. isariaeforme*). Other carbon sources such as glycogen or α-ketoglutaric acid appeared to be more or less inadequate.

Addition to the medium of various concentrations of witamins B_1 and B_2 or of complete set of vitamins did not result in the expected increase of growth of the mycelium of *P. claviforme* and *P. isariaeforme*. Both these species are therefore autoauxotrophic fungi (S c h o p f e r 1940), similarly as *A. giganteus* and *A. giganteus* mut. *alba*. It appeared that an addition of thiamine improved the shape of the developing coremia. This may be emphasized, as P a g e (1956) also established that

an addition of thiamine did not influence the development of *Pilobolus kleinii*, but thiamine was necessary for the elongation of sporangiophores. This may mean that thiamine exerts a particular and hitherto not quite clear influence on the elongation of the cells of fungi.

SUMMARY OF RESULTS

1. The section of *Penicillium clavigerum* consists of three species, namely *P. clavigerum* itself, *P. claviforme* and *P. isariaeforme* of these, only *P. clavigerum* grows well on simple synthetic media. The aim of the present study was to establish a synthetic medium suitable for the two remaining species.

2. Different modifications of the main medium used in previous investigations have been applied, yeast water being replaced by a complete set of amino acids and vitamins.

3. Several possible sources of nitrogen have been studied: it appeared that asparagine and ammonium tartrate were those most suitable for the moulds dealt with here.

4. Both above species assimilate carbon from sugars such as glucose, saccharose and fructose, but α-ketoglutaric acid and glycogen are unsuitable.

5. Addition of vitamins did not result in the expected increase of mycelial dry weight. On the other hand, thiamine influenced the shape of the developing coremia.

6. The composition of the medium suitable for *P. claviforme* is as follows: main Fries medium to which are added per 1 l of distilled water 1 mg thiamine, 5 g asparagine as source of nitrogen, 12.5 g glucose and 12.5 g fructose; for *P. isariaeforme*: main Fries medium to which are added per 1 l of distilled water 1 mg thiamine, a mixed source of nitrogen consisting of 5 g asparagine and 1 g ammonium tartrate, and a mixed source of carbon consisting of 12.5 g glucose, 6.25 g fructose, 6.25 g saccharose.

(Received: January 11, 1971)

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Badania nad wymogami pożywkowymi gatunków należących do sekcji *Penicillium clavigerum*

Streszczenie

1. Do sekcji *Penicillium clavigerum* należą trzy gatunki: *P. clavigerum*, *P. claviforme* i *P. isariaeforme*. Jedynie *P. clavigerum* rośnie doskonale na prostych pożywkach syntetycznych. Celem niniejszej pracy było określenie pożywki syntetycznej dla pozostałych dwu przedstawicieli sekcji.

2. Stosowano rozmaite modyfikacje pożywki zasadniczej używanej w badaniach wcześniejszych, zastępując wyciąg drożdżowy kompletem aminokwasów i witamin.

3. Spośród szeregu przebadanych związków azotowych najlepszym źródłem dla badanych grzybów była asparagina i winian amonu.

4. Jako źródło węgla oba gatunki wykorzystują takie cukry jak: glukoza, sacharoza, fruktoza, natomiast kwas α-ketoglutarowy czy glikogen są nieodpowiednie.

5. Dodatek witamin nie daje oczekiwanego dodatniego efektu na wzrost grzybni i suchej masy. Tiamina wpływa natomiast na kształt rozwijających się koremiów.

6. Skład odpowiedniej dla *P. claviforme* pożywki przedstawia się następująco: zasadnicza pożywka Fries z dodatkiem 1 mg tiaminy, 5a asparaginy jako źródło azotu, 12,5 g glukozy i 12,5 g fruktozy na litr wody destylowanej. Dla *P. isariaeforme* także pożywka stosowana przez Fries z dodatkiem 1 mg tiaminy, mieszanym źródłem azotu 5 g asparaginy i 1 g winianu amonu, mieszanym źródłem węgla — 12,5 g glukozy, 6,25 g fruktozy, 6,25 g sacharozy na litr wody destylowanej.