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SELECTION AND ISOLATION OF FILAMENTOUS FUNGI APPLICABLE IN PROTEIN ENRICHMENT OF CARBOHYDRATE RAW MATERIALS

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Key words: filamentous fungi, *Asp. oryzae*, starch, protein, biosynthesis.

Among the investigated filamentous fungi of genera *Aspergillus*, *Fusarium*, *Byssochlamys*, *Penicillium*, *Paelicomyces* and *Trichoderma*, the strain *Aspergillus oryzae A. or./6* was the most suitable for the biosynthesis of protein from starch-containing raw materials. Selection afforded four monocultures characterized by protein increase of 3.1-3.8 g/dm^3, and by protein yield (calculated per utilized starch) ranging from 24.7 to 31.1%; medium utilization fluctuated between 74.0-84.9%.

INTRODUCTION

In recent years, the agricultural and food industry by-products containing starch and cellulose have gained increasing interest of many research centers, representing still untapped raw material resources.

Filamentous fungi synthesizing both — amylolytic and cellulolytic enzymes — are microorganisms most often used in SCP production from starchy raw materials. *Aspergillus niger* [10, 11, 25] *A. fumigatus* [24], various species of the genera *Fusarium*, *Trichoderma* [2-6, 12, 24], and *Sporotrichum* pulverulentum [34] are used most often.

Sethi [28] has applied the strain *A. niger* (M1) to starch-rich (44% DM) mango stones and obtained a product containing 17.3% of crude protein and 12.1% of pure protein DM. Other authors [6, 18, 25] have used manioc, tapioca, jute or carob as raw materials treated with either *A. fumigatus*, *Sporotrichum thermophile* or *Paecilomyces*, and obtained fooder biomass containing 33-40% protein in dry mass. Sales i Manez [26] have received as many as 56.7% of protein using mixed cultures. Also Kożuchowa [19] has recommended the use of a mixed culture composed of *Trichosporon*

sp. and *Cephalosporium sp.*, affording 52-60% protein in dry mass of the product. Similar results have been obtained by Sakse [27] who used a peat hydrolysate, potato juice or wastes from citric acid production for bioconversion.

The present studies performed within the government program PR-4, were designed to isolate and select filamentous fungi applicable for protein biosynthesis from starchy raw materials. The suitability of the strains from the collection of the Institute of Fermentation Industry was checked, and a method of rapid strain preselection was developed.

MATERIALS AND METHODS

MICROORGANISMS

Amylolytic enzyme suythesizing filamentous fungi of genera *Aspergillus, Fusarium, Byssochlamys, Penicillium, Paecilomyces* and *Trichoderma*, from the culture collection of the Institute of Fermentation Industry were investigated. The strains were refrigerated at +4°C, and were reinculated every 6 months.

MEDIA

Pure cultures were maintained on a wort-agar medium of 8° Blg density, and on the Czapek-Dox medium with starch as the only carbon source (CzS).

The levels of protein biosynthesis and amylolytic enzyme synthesis were estimated in the following media:

(i) potato medium (PM): shredded potatoes — 125 g/dm^3, NH_4NO_3 — 4.5 g/dm^3, KH_2PO_4 — 4.0 g/dm^3, $MgSO_4 \cdot 7H_2O$ — 0.5 g/dm^3, pH = 5.2-5.3, starilization for 40 min at 118°C;

(ii) medium with rye grinding grain: rye grinding grain — 58.3 g/dm^3, NH_4NO_3 — 4.5 g/dm^3, KH_2PO_4 — 1.0 g/dm^3, $MgSO_4 \cdot 7H_2O$ — 0.5 g/dm^3, pH = 5.2-5.4, starilization for 60 min at 121°C.

Two other media were also used:

(iii) liquid Czapek-Dox medium: starch — 20 g/dm^3, $NaNO_3$ — 3 g/dm^3, $MgSO_4 \cdot 7H_2O$ — 0.5 g/dm^3, KCl — 0.5 g/dm^3, $FeSO_4$ — 0.01 g/dm^3;

(iv) natural medium according to Bernat 33: wheat bran — 33.4 g/dm^3, beet pulp — 6.4 g/dm^3, $CaCO_3$ — 0.16 g/dm^3, sterilization for 2 h at 0.5 atm.

ISOLATION AND SELECTION OF ACTIVE STRAINS

Isolation and selection of monocultures of the strains from the collection of the Institute of Fermentation Industry was done as follow: 5 cm^3

of sterile water were poured onto slants with sporulating cultures. After rinsing of the spores, the volume was made up to 10 cm^3 where upon suspension was further diluted till to obtain about 30 spores per 1 cm^3. For surface inoculation of selective medium CzS, 1 cm^3 of this suspension was used. After maturation of colonies Lugol solution was poured over the plates, and the colonies with the ratio of clearing zone radius (R) to colonyradius (r) exceeding 1.5 were isolated. The resulting monocultures were transferred to slants with CzS medium and refrigerated at +4°C.

CULTURE METHODS

The ability of the investigated strains to synthesize protein and amylolytic enzymes was determined in submerged cultures maintained on a rotary shaker (120 r.p.m.) in 500-cm^3 Erlenmeyer flasks containing 100 cm^3 of medium. The time of culture at 30°C ranged from 72 to 120 h; pH of the medium was 5.2-5.4. All samples were inoculated with 5 cm^3 of a suspension containing $1 \cdot 10^6$ spores/cm^3.

ANALYTICAL METHODS

The following determinations were performed:

a. for microorganisms

— morphological traits of mycelium and colonies [29, 30, 32],

— specific growth rate in liquid medium, calculated from the formula (1, 2)

$$u = \frac{0.693}{g}$$

where g is the generation time,

— generation time, g, calculated from the formula (1, 2)

$$g = \frac{t \cdot \log 2}{\log b - \log a}$$

where a — initial content of biomass in g DM, b — final content of biomass in g DM, t — observation period (h),

— dry mass content [22],

— total protein content by the Kjeldahl method [31, 33], using the Kjell-Foss Automatic 16210 apparatus,

— ammonium nitrogen content [31],

— aflatoxin content by TLC [21];

b. for post-culture liquid

— alpha-amylase activity in AS units according to Klimowski and Rodziewicz [23]; this assay consists in measurement of the time of starch hydrolysis to dextrins which no more give a colour reaction with iodine

in the presence of alpha-amylase; the activity units AS represent the amount of enzyme which hydrolyses 1 g of starch to dextrins in 1 h at 30°C;

— total sugar content after hydrolysis, determined colorimetrically in the presence of DNS [22].

MATHEMATICAL TREATMENT

The results were statistically treated Elandt, .

The applied methods were charged with the following errors:

— amylolytic activity assay — 2.5%,

— protein determination in the Kjell-Foss apparatus — 1%,

— total sugar content assay (after hydrolysis) using DNS — 1.88%.

The protein content was calculated from $(N_{Kjeld.} - N_{amon.}) \cdot 6.25$, and the result was converted to biomass DM. Protein yield was calculated as follows:

$$\text{Yield } (\%) = \frac{\text{protein increase in g} \cdot 100}{\text{DM loss or added starch or else utilized starch.}}$$

RESULTS

PRESELECTION OF STRAINS

Preselection concerned filamentous fungi strains characterized by the ability to synthesize amylolytic enzymes and kept in the collection of the Institute of Fermentation Industry: *Aspergillus oryzae* A. or./5, A. or./6, A. or./7, A. or./9; *A. versicolor* A. v./1, *A. niger* A. n./54, *A. candidus* A. c. /4, *Fusarium avenaceum* F. a./1, *F. scirpi* F. s./1, *Byssochlamys fulva* B. f./1, *Penicillium roseopurpurogenum* P. rp./1, *Paecilomyces varioti* Pel. v./1, Pel. v./2 and *Trichoderma lignorum* Tch. l./1, Tch. l./2.

The results are given in Table 1. The protein content in the biomass of strains ranged from 34.87 to 46.18% DM and their amylolytic activity — from 5.0 to 65.3 AS U/100 cm^3. The inter-strain differences in the growth rate were also remarcable. Generation times varied between 172.3-60.4 h. Owing to their very low amylolytic activity, all strains other than *Aspergillus oryzae* were eliminated. Out of the four strains of this species, A. or./6 was selected for further studies, since it displayed an adequate amylolytic activity, protein content in biomass of 45.22% DM, as well as a relatively high growth rate.

The amount of inoculum clearly affected the amylolytic enzyme content in the post-culture liquid. An increase in inoculum density from 10^5 to 10^6 conidia/cm^3 resulting in an activity rise of 16 to 40%, depending

Table 1. Preselection of strains

Strain	Amylolytic activity (AS μ/100 cm³)	Protein (% DM)	Generation time (g/h)	Specific growth rate (μ/h⁻¹)
Aspergillus oryzae *A.or*/5	65.3	46.18	140.0	0.005
A.or/6	65.3	45.22	61.2	0.011
A.or/7	43.8	43.65	93.3	0.007
A.or/9	51.0	39.22	95.9	0.007
Aspergillus versicolor *A.v*/1	5.0	42.52	115.4	0.006
Aspergillus candidus *A.c*/4	5.0	36.74	111.2	0.006
Aspergillus niger *A.n*/54	5.0	42.96	154.8	0.004
Fusarium avenaceum *F.a*/1	5.0	34.87	132.8	0.005
Fusarium scirpi *F.s*/1	9.4	38.48	123.2	0.006
Byssochlamys fulva *B.f*/1	5.0	40.36	110.0	0.006
Penicillium roseopurpurogenum *P.rp.*/1	6.8	43.87	172.3	0.004
Paecilomyces varioti *Pel.v*/1	8.8	37.83	60.4	0.011
Pel.v/2	5.0	35.69	138.3	0.005
Trichoderma lignorum *Tch.l*/1	5.0	34.93	134.7	0.005
Tch.l/2	5.0	35.51	151.0	0.005

The results represent the mean values fortripl cate cultures

on the strain. Therefore in all subsequent experiments suspensions containing 10^6 conidia/cm³ were applied.

ISOLATION OF MONOCULTURES

Before undertaking monoculture isolation, we developed a method for their preselection. Relying on literature data [1, 15, 30], we assumed that strains with higher enzymatic activity exhibit less intense growth and sporulation. The greatest activity ought to be demonstrated by strains which on the selective medium are characterized by a wide clearing zone (R) and small colony radius (r), i.e. those with high R/r ratios.

To check this assumption we isolated active monocultures of strain A. or./6 by inoculating a conidia suspension containing 20-40 conidia/1 cm³ [13]. As selective medium, the Czapek-Dox medium with starch as the only carbon source (CzS) was used, and Lugol solution served as developer. Incubation was carried out for 120 h at 30°C, and monocultures with ratio R/r 1.2 were isolated.

Prior to the monoculture activity assay, the dynamics of amylolytic enzyme synthesis by the parent strain A. or./6 in shaker cultures [16, 17] were determined. The results are shown in Fig. 1.

The amylolytic activity of strain A. or./6 was highest after 120 h on the potato medium. The activities on the other media were lower by 26% (natural medium containing wheat bran and beet pulp) and 58% (CzS);

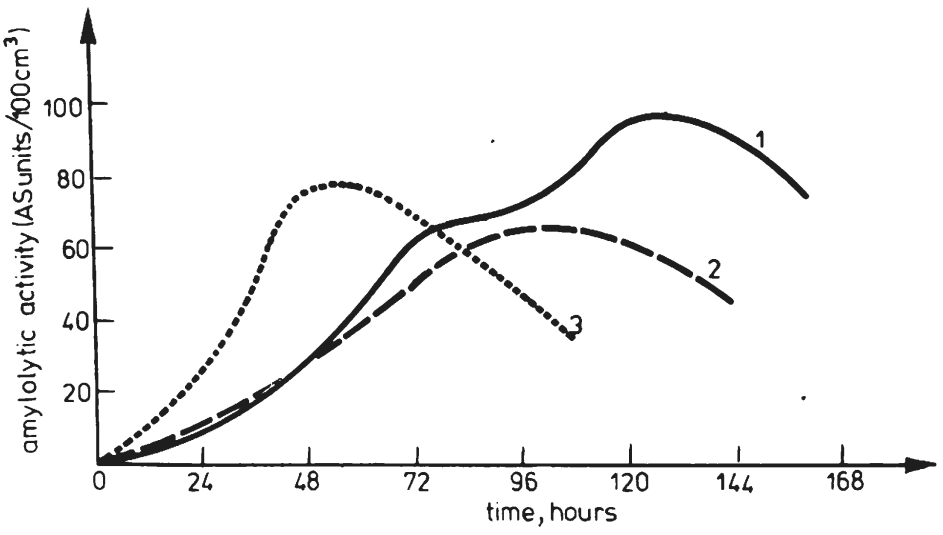

Fig. 1. Dynamics of biosynthesis of amylolytic enzymes in shaker cultures; 1 — potato medium, 2 — Czapek-Dox medium with starch, 3 — medium containing wheat bran and beet pulp

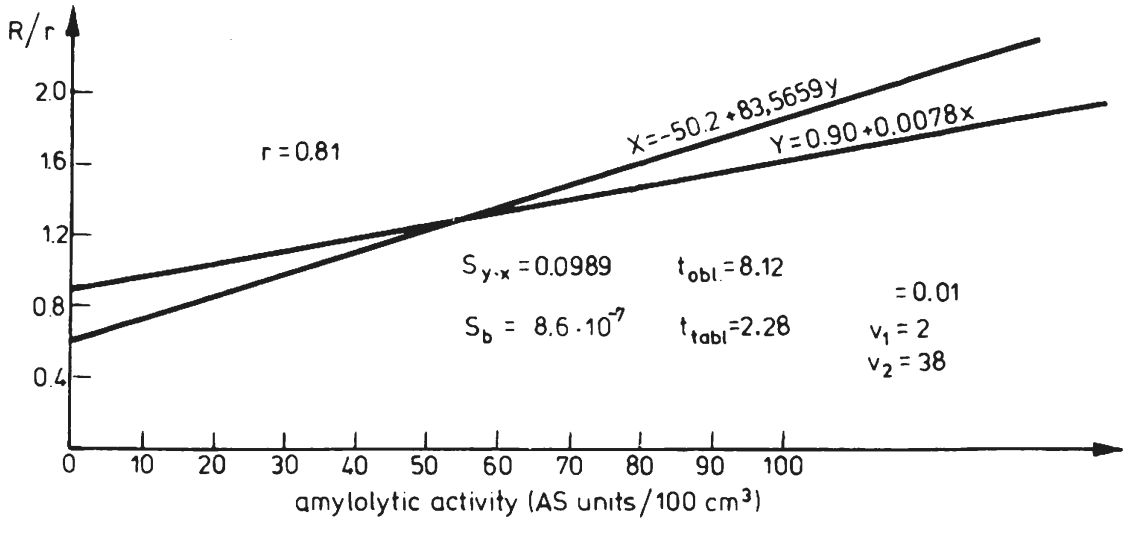

Fig. 2. Correlation between R/r and amylolytic activity

therefore in the further enzymic activity assays in monocultures the potato medium was applied.

The correlation between the amylolytic activity and the R/r ratio is ilustrated in Fig. 2. Among the 40 monocultures isolated, as many as six, i.e. 15% exhibited an amylolytic activity exceeding 91 AS U/100 cm^3, 18 monocultures (i.e. 45%) an activity of 71-90 AS U/100 cm^3, and 12 (30%) — an activity of 51-70 AS U/100 cm^3. Statistical treatment of the results pointed to a relatively strong positive correlation between the amylolytic activity and the R/r ratio ($r = 0.81$). The regression was highly significant, since calculated value of $t = 8.12$ was higher than that from the tables (2.28). The suitability of the applied preselection method was confirmed by the low values of the standard deviation of the regression $S_{y.x} = 0.0989$.

According to the present results the Czapek-Dox medium with starch as the only carbon source and Lugol solution as developer may be used

to isolate active strains synthesizing amylolytic enzymes. When monocultures with ratio R/r 1.5 are isolated, strains with an activity exceeding 65 AS U/100 cm^3 can be expected.

CHARACTERIZATION OF THE MONOCULTURES OBTAINED

For characterization of the selected monocultures, at first the dynamics of biosynthesis of protein and amylolytic enzymes in one of the active monocultures A. or./6/9 were determined. The results are recorded in Fig. 3. The amount of biomass, and hence also of protein, amounting to 13.4 g DM/dm^3 and 5.67 g/dm^3, respectively, was greatest after 72 h of culture, whereupon the biomass content gradually decreased, owing to the beginning of mycelium autolysis. This process became gradually more intense (microscopic observations) till 120 h of culture when the amylolytic activity attained its peak. Therefore the cultures intended for the determination of the protein biosynthesis level kept for 72 h.

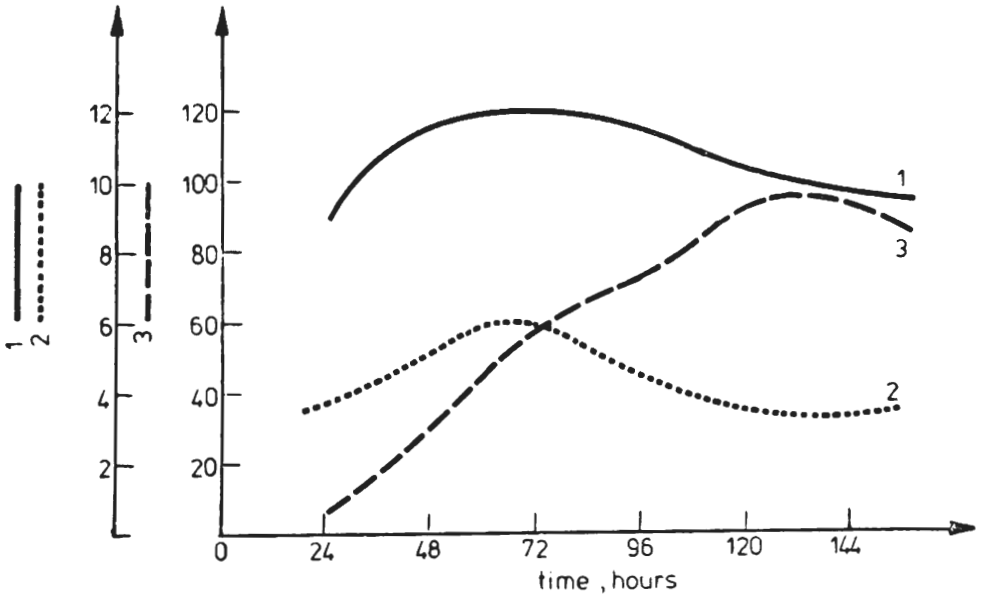

Fig. 3. Dynamics of protein and amylolytic enzymes biosynthesis by *A. oryzae*/6/9 in shaker cultures; 1 — biomass content, 2 — protein content in biomass, 3 — amylolytic activity

For selection of strains characterized not only by an ability to grow on starchy raw materials but also by high protein content in DM and a short generation time, all these parameters were assayed in monocultures displaying an amylolytic activity greater than 62 AS U/100 cm^3. The CzS medium was used, and the results are given in Table 2.

It was found that out of the 15 best monocultures as six, i.e. 40% were characterized by a protein content in biomass lower than 39.9% DM. In four monocultures, i.e. 26.7% the protein content ranged from 40.0 to 44.9% DM, while five monocultures (33.3%) had a protein content in bio-

Table. 2. Protein content, amylolytic activity and growth rate of monocultures

Monoculture *A.or*(6)x	Protein content (%DM)	Amylolytic activity (AS μ/100 cm³)	Generation time (g/h)	Specific growth rate, (μ/h⁻¹)
2	49.52	83.33	20.00	0.035
5	47.58	93.75	20.94	0.033
6	49.52	90.36	23.11	0.030
7	34.52	96.77	21.37	0.032
8	47.83	76.97	19.03	0.036
9	35.47	90.99	19.62	0.035
11	45.43	81.14	23.55	0.029
13	44.09	85.78	26.28	0.026
15	42.32	86.97	21.15	0.033
16	38.34	86.97	19.74	0.035
21	43.89	90.99	20.06	0.035
23	35.36	79.16	17.20	0.040
24	43.42	79.16	24.33	0.028
37	36.99	93.75	40.17	0.017
38	35.78	83.33	42.75	0.016

mass, amounting to 45% DM. There was no correlation between the amylolytic activity and protein content (r = 0.265). The monocultures also exhibited differences in the growth rate, as indicated by the generation times amounting e.g. to 47.4 h for strain A. or./6/36 and to only 17.07 h for strain A. or./6/18.

For assessment of the suitability of the various monocultures for protein enrichment of starchy raw materials, the absolute protein increase per medium unit and protein yield was determined in shaker cultures. The results are given in Table 3. In four monocultures (19.06%) the protein increase per 1 dm³ was relatively high amounting to 3.22-3.48 g. It is noteworthy that the monocultures denoted A. or./6/9 and A. or./6/16 displayed a higher growth rate and lower protein content in DM (35.47 and 38.34% DM, respectively) while the remaining two monocultures, A. or./6/2 and A. or./6/8 displayed a relatively high protein content (47.83% and 49.52% DM, respectively), but their growth rate was lower. A smaller protein increase (2.50-2.99 g/dm³) was shownby four monocultures (36.36%), whereas the respective range was 2.00-2.49 g/dm³ for three other ones (27.27%).

In the case of the four best monocultures, 25.67 to 27.12 kg of potatoes (10.85% of starch) were needed to obtain 1 kg of protein in the medium, whereas 35.92 to 38.82 kg of potatoes were required for an increase of 1 kg protein. For these cultures the yield of protein biosynthesis calculated per DM loss was 30.85-45.71%, and calculated per starch it was 23.74-25.66%. The present results indicate that the monocultures A. or./6/2, A. or./6/8, A. or./6/9 and A. or./6/16 may be used for protein enrichment of starchy raw materials.

Table 3. Protein increase in the medium and yield of biosynthesis in several monocultures

Monoculture *A. or.* (6)x	Biomass		Protein increase in 1 dm^3 of medium (%)	Protein yield calculated per:			Raw material utilization per 1 kg	
	dry mass (g/dm^3)	protein (g/dm^3)		DM loss (%)	added starch (%)	added raw material (%)	protein in medium (kg)	protein increase (kg)
2	9.84	4.87	3.48	30.85	25.56	2.78	25.67	35.92
5	8.12	3.86	2.47	19.54	18.21	1.97	32.38	50.61
6	8.48	4.19	2.80	22.80	20.64	2.24	29.83	44.64
8	9.83	4.70	3.31	30.28	24.41	2.64	26.60	37.76
9	13.41	4.75	3.36	45.71	24.77	2.68	26.32	37.20
11	9.00	4.08	2.69	22.87	19.83	2.15	30.64	46.47
13	8.56	3.77	2.38	19.50	17.55	1.90	30.16	52.52
15	8.03	3.39	2.00	15.71	14.74	1.60	36.87	62.50
16	12.03	4.61	3.22	36.88	23.74	2.57	27.12	38.82
21	9.05	3.97	2.58	22.03	19.02	2.06	31.48	48.45
24	8.49	3.69	2.30	18.74	16.96	1.84	33.88	54.35

The medium contained: 20.76 g DM/dm^3; 13.56 g starch/dm^3; 1.39 g potato protein/dm^3

Table 4. Yield of protein biosynthesis by four active monocultures on a medium with rye grinding grain

Monoculture *A.or.* (6)x	Biomass		Protein increase in 1 dm^3 of medium (g)	Protein yield calculated per:			Raw material utilization per 1 kg	
	dry mass (g/dm^3)	protein (g/dm^3)		DM loss (%)	added starch (%)	added raw material (%)	protein in medium (kg)	protein increase (kg)
2	27.27	11.59	7.06	29.10	17.45	13.70	5.03	8.26
8	26.82	11.28	6.75	27.32	16.68	13.10	5.17	8.63
9	27.71	11.46	6.93	29.09	17.45	13.45	5.09	8.41
16	23.30	9.80	5.27	18.66	13.03	10.23	5.95	11.06

The medium with grinding contained: 51.53 g DM/dm^3; 40.46 g starch/dm^3; 4.53 g protein/dm^3

As the next stage of the experiments, the suitability of the medium with rye grinding grain for the above-mentioned four monocultures was evalueted. The results are given in Table 4. Comparision of this data with the results shown in Table 3 indicates that the protein increase in the investigated monocultures maintained on the medium with the rye grinding grain (5.27-7.06 g/dm^3) was about 80% bigger than in case of the potato medium. On the other hand the protein yield calculated per added starch was about 38% lower than that obtained using the potato medium, and ranged from 13.03 to 17.45% (the respective numbers for the potato medium 23.75 and 27.93%). In comparision to the potato medium, in case of this medium much less raw material was needed to obtain 1 kg protein in the medium (5.03 kg for A. or./6/2 and 5.95 kg for A. or./6/16; for a 1 kg increase in protein, from 8.26 to 11.06 of raw material were required.

The ability of the four best strains (A. or./6/2, A. or./6/8, A. or./6/9 and A. or./6/16), selected for further studies, to synthesize aflatoxins B_1, B_2, G_1 and G_2 was checked. These strains were found not to synthesize aflatoxins under the experimental conditions applied.

EFFECT OF STORAGE TIME ON THE PROTEIN BIOSYNTHESIS ABILITY

The stability of the ability to enrich starchy raw materials in protein was checked after 6 and 12 months of storage at +4°C. The results shown in Table 5 indicate that this ability was satisfactory in the studied strains. The fluctuations of this ability were smallest for the monocultures of A. or./6/9 and A. or./6/16.

The results were slightly different for the remaining two strains characterized by a lower growth rate and higher protein content in DM (47.83 and 49.52, respectively). In the case gradual increase in the growth rate was paralleled by a slight drop in protein content in DM. However, this fact had no effect on both — the increase in protein in the medium and protein yield calculated per added starch. Table 6 presents the yield of protein biosynthesis and the degree of medium utilization by the four monocultures after 16 months of storage. The results shown in Table 5 and 6 point to a slight drop in protein yield calculated per added starch, and to medium utilization of about 80%. The protein increase per 1 dm^3 of medium was 3.11-3.78 g and protein yield calculated per utilized starch was 24.74-31.06%.

DISCUSSION OF RESULTS

The avaibility of rapid growing fungus strains characterized by a high yield of protein from the given raw materials is the main prerequisite

Table 5. Effect of storage time on protein biosynthesis by the monocultures

Monoculture *A.or.* (6)x	Storage time (months)	Biomass		Protein increase in 1 dm³ of medium (g)	Protein yield calculated per:			Raw material utilization per 1 kg	
		dry mass (g/dm³)	protein (g/dm³)		DM loss (%)	added starch (%)	added raw material (%)	protein in medium (kg)	protein increase (kg)
2	0	9.84	4.87	3.48	30.85	25.66	2.78	25.67	35.92
	6	12.81	5.26	3.84	46.10	27.29	3.07	23.76	32.55
	12	13.54	5.35	3.93	51.57	27.93	3.14	23.36	31.81
8	0	9.83	4.70	3.31	30.31	24.41	2.65	26.60	37.76
	6	12.64	4.84	3.42	40.24	23.53	2.74	25.83	36.55
	12	13.08	5.08	3.66	45.41	26.01	2.93	24.61	34.15
9	0	13.41	4.75	3.36	45.71	24.78	2.69	26.32	37.20
	6	13.90	5.32	3.90	53.87	27.72	3.12	23.50	32.05
	12	13.23	5.24	3.82	48.29	27.15	3.06	23.85	32.72
16	0	12.03	4.61	3.22	52.80	23.75	2.58	27.12	38.82
	6	12.69	5.17	3.75	44.38	26.65	3.00	24.18	33.33
	12	12.88	5.12	3.70	44.79	26.30	2.96	24.41	33.78

The potato medium contained: at time 0:20.76 g DM/dm³, 13. 56 g starch/dm³, 1.39 g protein/dm³; after 6 and 12 months — 21.14 g DM/dm³, 14.07 g starch/dm³, 1.42 g protein/dm³

Table 6. Protein biosynthesis yield in the selected monocultures and their degree of medium utilization after 16 months of storage

Monoculture *A.or.* (6)x	Utilized starch		Biomass		Protein increase in 1 dm^3 of medium (g)	Protein yield calculated per:		
	(g/dm^3)	(%)	dry mass (g/dm^3)	protein (g/dm^3)		added starch (%)	utilized starch (%)	mass loss (%)
2	12.30	80.6	14.17	5.63	3.78	24.77	30.70	95.71
8	11.27	73.9	13.90	5.35	3.50	22.93	31.06	92.83
9	12.96	84.9	13.89	5.32	3.47	22.74	26.77	74.76
16	12.57	82.4	12.64	4.96	3.11	20.38	24.74	62.20

The potato medium contained: 17.67 g DM/dm^3; 15.26 g starch/dm^3; 1.85 g protein/dm^3

for the use of starchy products for obtainment of feed preparations. The content of pure protein in the resulting preparations should at least twice exceed that in the raw materials.

This condition is satisfied by the four selected A. oryzae strains which after 72 h of culture at 30°C give a product containing 35.5-40.8% of protein in DM, i.e. 5.8 times more as compared with the initial medium (6.7% protein in DM). Similar results have been obtained by Drouliscos et al. [8], Khor [18], and Reade and Gregory [25], who used manioc, tapioca etc. as raw materials treated with *A. fumigatus*, *Sporotrichum thermophile* and *Paecilomyces sp.* Lower protein contents in the products have been reported by Raimboult et al [24] who used *A. niger* and potato medium (20% protein in DM of the product), and by Sethi [18] who obtained 12.1% of protein, also using *A. niger*. A considerably higher protein content in the product, amounting to 51-68% of DM, has been found by Sakse [27]; his results, however, are uncomparable since he does not specify the strain used.

CONCLUSIONS

The present results lead to the following conclusions:

1. Among the investigated filamentous fungi of genera *Aspergillus*, *Fusarium*, *Byssochlamys*, *Penicillium*, *Paecilomyces* and *Trichoderma*, *Aspergillus oryzae* (*A. or.*/6) grew best on starchy raw materials.

2. Further selection afforded four monocultures of *A. oryzae A. or.*/6, characterized by good growth on the carbohydrate medium and by a high protein content in the biomass. The protein increase was 3.1-3.8 g/dm^3, protein yield calculated per utilized starch was 24.7-31.1%, and the utilization of starch from the medium amounted 74-84.9%.

3. The protein biosynthesis ability of the monocultures *A. or.*/6/2, *A. or.*/6/8, *A. or.*/6/9 and *A. or.*/6/16 remainded unchanged over time. After 16 months of storage the drop in the protein increase in 1 dm^3 of medium and the decrease in protein yield were only slight (5%).

4. The Czapek-Dox medium containing starch as the only carbon source and Lugol solution used as a developer can be used for isolation of active amylolytic cultures. When the ration of clearing zone diameter (R) to colony diameter (r) exceeds 1.5, the presence of strains with amylolytic activity greater than 65 AS U/100 cm^3 can be expected.

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PRÓBA SELEKCJI I IZOLACJI GRZYBÓW STRZĘPKOWYCH PRZECHOWYWANYCH W KOLEKCJI IPF PRZYDATNYCH DO WZBOGACANIA SUROWCÓW WĘGLOWODANOWYCH W BIAŁKO

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Streszczenie

Stwierdzono, że spośród przebadanych grzybów strzępkowych należących do rodzajów: *Aspergillus*, *Fusarium*, *Byssochlamys*, *Penicillium*, *Paecilomyces* i *Trichoderma* najbardziej przydatny do biosyntezy białka z surowców skrobiowych okazał się szczep *Aspergillus oryzae* oznaczony symbolem *A.or.*/6 (tab. 1).

Opracowano szybką metodę selekcji wysokowydajnych szczepów wytwarzających enzymy amylolityczne opierającą się na określeniu stosunku średnicy rozjaśnienia (R) do średnicy kolonii (r) na podłożu Czapek-Doxa z dodatkiem zhydrolizowanej do dekstryn skrobi jako jedynym źródłem węgla oraz płynem Lugola jako wywoływaczem. Otrzymane wyniki zweryfikowano metodami statystycznymi (rys. 2). Dla monokultury *A.or.*/6/9 określono dynamikę biosyntezy białka i enzymów amylolitycznych stwierdzając, że najwięcej białka uzyskuje się w 72 h hodowli, a najwyższą aktywność w 120 h hodowli (rys. 3).

W wyniku przeprowadzonej selekcji szczepu *A.or.*/6 otrzymano 4 monokultury o bezwzględnym przyroście białka wynoszącym 3,22-3,48 g/dm^3, wydajność procesu w przeliczeniu na ubytek suchej masy wahała się od 30,85 do 45,71%, a w przeliczeniu na skrobię od 23,74 do 25,66% (tab. 3). Następnie sprawdzono przydatność wyselekcjonowanych 4 monokultur do wzrostu na podłożu ze śrutą żytnią i stwierdzono, że bezwzględny przyrost białka był większy o ok. 80% niż na pożywce ziemniaczanej, natomiast wydajność białka w przeliczeniu na wprowadzoną skrobię była niższa o ok. 38% niż w przypadku podłoża ziemniaczanego (tab. 4).

W tab. 5 i 6 przedstawiono wpływ czasu przechowywania na zdolność do biosyntezy białka badanych 4 monokultur i stwierdzono, że była ona zadowalająca. Po 16 miesiącach przechowywania przyrost białka w 1 dm^3 podłoża wahał się w granicach 3,11-3,78 g, a wydajność białka w przeliczeniu na wykorzystaną skrobię 24,74-31,06%.