A C T A A L I M E N T A R I A P O L O N I C A
Vol. XII (XXXVI), No. 3—4 1986

HELENA STROBIŃSKA
HELENA OBERMAN

SELECTION OF YEASTS IN STARCHY MEDIA AND EVALUATION OF THEIR GROWTH IN STARCH-CONTAINING MEDIA

Institute of Fermentation Technology and Microbiology,
Technical University, Łódź

Key words: *Candida tropicalis*, *Candida mogii*, starch degradation, growth stimulators.

Yeast strains *Candida tropicalis* and *C. mogii* capable of metabolizing starch were isolated from starchy media. This ability of the yeasts was stimulated by starch concentration, pH value and the presence of growth stimulators in the medium.

INTRODUCTION

Various microscopic fungi, including species of the genera *Candida*, *Torulopsis*, *Endomycopsis*, *Hansenula* and *Monilia*, may be used in the production of food protein [2, 4, 5, 11, 13-15]. The protein-producing microorganism ought to have a well developed respiratory enzyme complex, should be able to convert carbohydrates to protein, to synthesize vitamins and enzymes, and be resistant to changes in the culture medium composition. It is also desirable that the produced protein have a correct amino acid composition and, if possible, that it be heavily augmented with exogenous lysine and methionine amino acids [7].

Predominant in Poland is the production of yeast protein from molasses. The varying availability of this raw material prompts the search for other cheap and easily available substrates. The numerous studies of effective utilization of cereal and potato mashes as fodder go in this direction.

Starch, being a cheap and nontoxic carbon source, is among the important potential substrates for the production of food protein. However, an industrial-scale production of this protein requires chemical or enzymatic starch hydrolysis. To eliminate the process of hydrolysis which prepares the starch medium for biomass production, it is necessary to use

microorganisms of high amylolytic activity [13]. This task is simple enough in the case of bacteria, but the use of microscopic fungi requires a number of basic investigations.

In this research we attempted to select yeasts of an increased amylolytic activity from natural media and to test their usefulness in starch utilization in synthetic and natural media.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

The experiments were performed with two strains, *Candida tropicalis* and *C. mogii*, selected from among 17 yeast strains isolated in starchy media (refined, slime and potato starch).

CULTURE MEDIA AND CONDITIONS OF CULTURE

The yeast strains were cultured on agar slants of YPG and YPS media (Table 1) at 30°C for 48 h. They were stored at 4°C and inoculated every 10 days, serving as inoculum for plate and submerged cultures. The studied yeasts' ability to utilize starch was determined in the Mo medium in which starch was the only source of carbon. The composition of the Mo medium is given in Table 1.

Table 1. Culture media

Medium	Application
Y.P.G. (cf. [1])	Diagnostics, selection and storage of strains
Y.P.S. (cf. [2])	
Mo standard medium (% w/v)*)	Strain selection, study of amylolytic ability
$(NH_4)_2SO_4$ 0.30	
KH_2PO_4 0.10	
$MgSO_4 \cdot 7H_2O$ 0.05	
starch 0.5-2.0	
yeast extract 0.01-0.10	
thiamine 5 μg/dm³	
biotin 10 μg/dm³	
Potato medium (% w/v)*)	Characteristic of *C. tropicalis* and *C. mogii* growth
$(NH_4)_2SO_4$ 0.30	
KH_2PO_4 0.10	
$MgSO_4 \cdot 7H_2O$ 0.05	
disintegrated potatoes 10	
tap water pH 5.5	

*, w/v = weight per volume

SELECTION OF AMYLOLYTICALLY ACTIVE YEASTS

The preselection of highly amylolytic yeasts was performed in two stages according to the scheme in Fig. 1. The first stage was plate culture in which selection was performed on the basis of the size of the formed

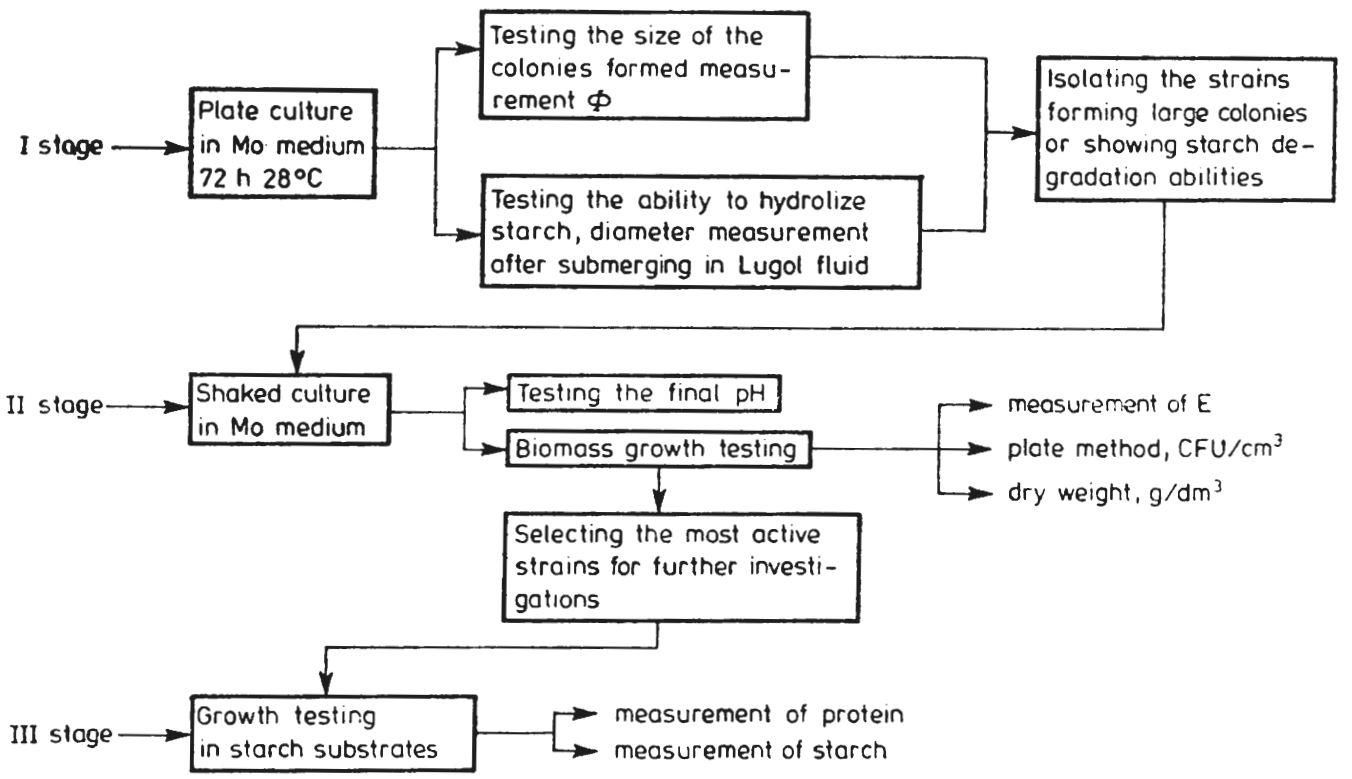


Fig. 1. Stages in the selection of yeasts utilizing starch

colonies and of the zone of starch decomposition revealed by Lugol fluid. The second stage of selection was performed on shaker cultures with the Mo medium. The criterion for evaluating the strains was biomass increase after 72 h of culture. Biomass yield was characterized by the coefficient Y_p, i.e. the ratio of yeast dry mass (g) to the amount of introduced substrate (g) [8, 13].

TAXONOMIC ANALYSIS OF STARCH ISOLATES

The yeasts were identified according to diagnostic tests due to Lodder [10] and Barnett [3]. These included classical taxonomic analyses determining the morphological, physiological and biochemical properties of the cells, and characterizing growth on standard media as well as reproduction.

GROWTH OF *CANDIDA TROPICALIS* AND *C. MOGII* ON SYNTHETIC MO MEDIUM AND ON POTATO MEDIUM

The strains were cultured in 500-cm³ flasks with 50 cm³ of medium inoculated with 10% v/v standardized suspension of yeast from the end

of the logarithmic growth phase. The cultures were shaken at 220 r.p.m. and 30 mm amplitude, at 30°C for 72 h.

The following was assessed in the culture media:

— number of cells by the plate method, giving the result as the number of units capable of colony growth (CFU/cm^3) or biomass by the weight method (g/dm^3) or indirectly by the spectrophotometrical method measuring E at $\lambda = 530$ nm [16];

— protein content by the Kjeldahl method [16];

— reducing substances and starch by the Luff-Schoorl method [16].

Also determined were values of the basic parameters of growth: specific growth rate μ, generation period T as the time of biomass doubling, and yield coefficient Y_p [8, 13].

RESULTS AND DISCUSSION

SELECTION OF AMYLOLYTIC STRAINS FROM NATURAL MEDIA

Yeasts with amylolytic capabilities were isolated from samples of slime, refined and potato starch. In the first two analysed starches there occurred strains of mesophilic and psychrophilic yeasts numbering from $2.6 \cdot 10^5$ to $7.2 \cdot 10^6$ CFU/g; in stored potato starch the number of yeast cells ranged from 5.10 to $1.4 \cdot 10^3$ CFU/g (Table 2). The yeast strains belonged to the genera *Saccharomyces*, *Candida*, *Torulopsis Trichosporon* and *Rhodotorula* (Table 2). Five strains included in the genus *Candida* on the basis of preliminary diagnostic tests were chosen for the selection studies. The results of the selection are given in Table 3.

As can be seen, the populations isolated from starchy media display various abilities to utilize starch. In plate cultures they formed colonies with diameters (equal to the zone of starch decomposition) of 9-13 mm; in shaker cultures their multiplication ranged from $3.85 \cdot 10^7$ to $1.1 \cdot 10^9$

Table 2. Yeast strains occurring in the starches

Product	No of yeast cells CFU g	Genera
Slime starch from hydrocyclone	$3.3 \cdot 10^5$-$1.2 \cdot 10^6$	*Candida*
Slime starch from separator	$3.9 \cdot 10^6$-$7.2 \cdot 10^6$	*Candida* *Trichosporon* *Rhodotorula*
Refined starch	$2.6 \cdot 10^5$-$2.8 \cdot 10^6$	*Candida* *Torulopsis* *Saccharomyces*
Potato starch	$5.0 \cdot 10^1$-$1.4 \cdot 10^3$	*Candida*

Table 3. Growth of yeast isolates in plate and shaker cultures on standard medium Mo supplemented with 2% starch

Yeast	pH after culture	Dry mass (g/dm³)	CFU/cm³	Colony diameter (mm)
Candida 1	4.5	0.58	$4.04 \cdot 10^7$	12
Candida 2	4.2	1.06	$3.85 \cdot 10^7$	10
Candida 3	4.3	0.92	$1.72 \cdot 10^8$	13
Candida 4	3.0	2.26	$1.10 \cdot 10^9$	11
Candida 5	3.2	3.24	$9.70 \cdot 10^8$	9

cells/cm³. Biomass production in the Mo medium varied in the range from 0.58 to 3.24 g/dm³ (Table 3). The most intense starch utilisation was stated by strains *Candida* 4 and *Candida* 5, and these were selected for further study.

TAXONOMIC STUDIES OF *CANDIDA* 4 AND *CANDIDA* 5

The yeasts in question ferment glucose, saccharose and maltose, and they assimilate various carbohydrates, organic acids and alcohols; they use starch as the only source of carbon and energy. The studied strains lack the enzyme urease and this fact ruled out the possibility of their belonging to *Heterobasidiomycetes* and *Schizosaccharomyces* at the stage of preliminary classification [9, 12]. They may grow in a wide range of temperatures ranging from 4 to 37°C. In young cultures the cells are arranged singly and their gemmation is polar, while in older cultures they combine to form chains of 2-6 cells. They form pseudomycelia and mycelia.

Comparing the obtained diagnostic results with tests according to Lodder and Barnett it was possible to identify the strains as *Candida tropicalis* and *Candida mogii* [3, 10].

EFFECT OF ENVIRONMENTAL FACTORS ON THE ABILITY OF *CANDIDA TROPICALIS* AND *C. MOGII* TO METABOLIZE STARCH

The kinetics of yeast growth were determined in standard medium Mo

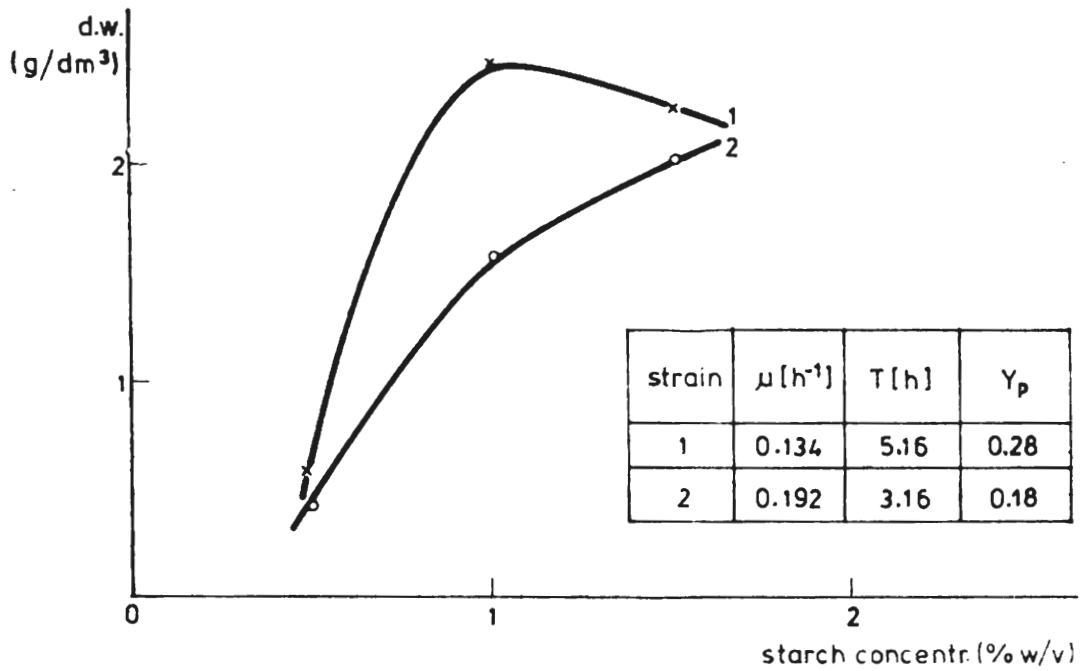
1) with pH values ranging from 4.5 to 5.8,

2) in the presence of various starch concentrations (0.5-1.5% w/v), and

3) of growth stimulating factors.

The stimulators were yeast extract, thiamine and biotin (Table 1). The results of these studies are shown in Figs 2-5.

As we can see in Fig. 2, the yeasts isolated from various materials displayed amylolytic capabilities. This property was especially prominent



strain	μ [h⁻¹]	T [h]	Y_p
1	0.134	5.16	0.28
2	0.192	3.16	0.18

Fig. 2. Effect of starch concentration on biomass yield of *Candida tropicalis* (1) and *C. mogii* (2) in Mo medium

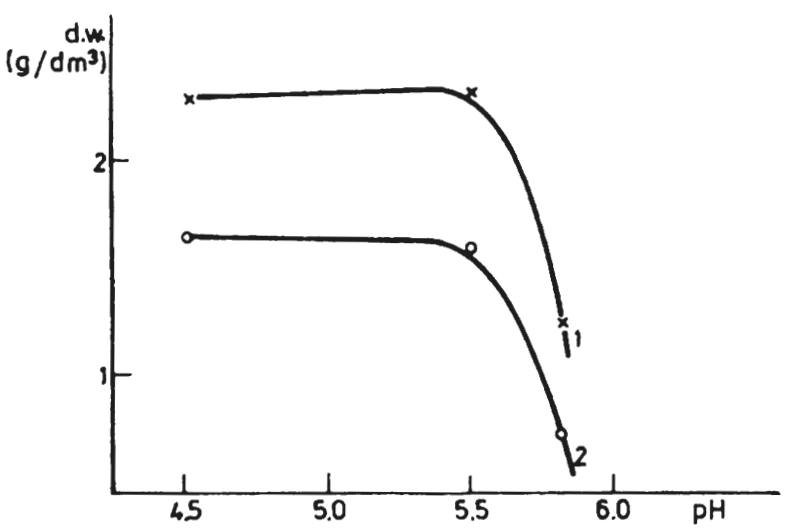
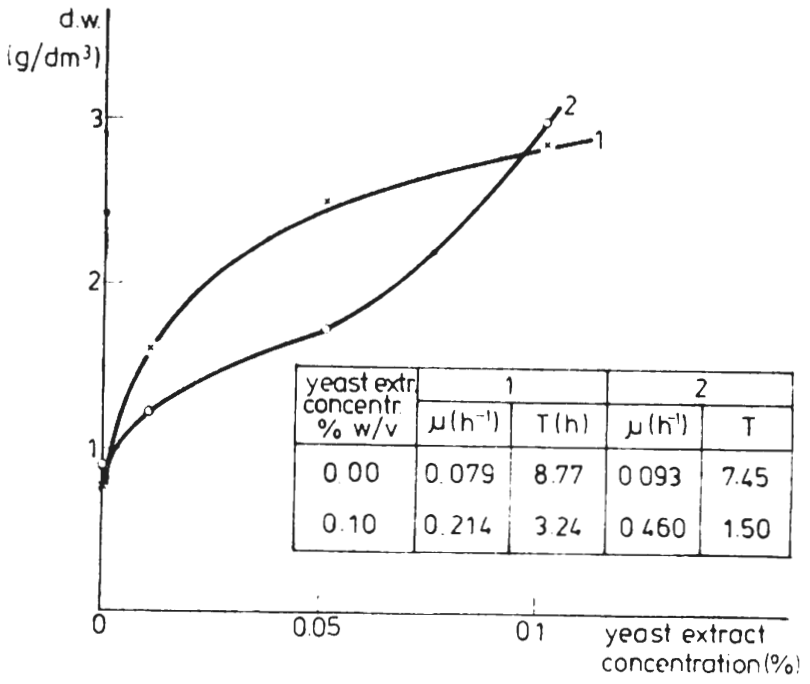


Fig. 3. Biomass production by *C. tropicalis* (1) and *C. mogii* (2) at different pH values in Mo medium with 1% starch

at 1% concentration of starch as the only carbon source in the standard medium Mo. The strains *C. tropicalis* and *C. mogii* exhibited the most favourable growth parameters at this concentration. The specific growth rate μ attained values of 0.134 h^{-1} to 0.192 h^{-1}, the generation period T was 3.6-5.1 h, and the yield coefficient Y_p = 0.18-0.28. The *C. tropicalis* yeasts described by Spencer [13] also utilized starch, and their Y_p coefficient was 0.234. The studied *C. tropicalis* isolate was thus more active in the Mo medium with optimum starch concentration (Fig. 2). The results shown in Fig. 3 indicate that the pH of the culture medium affects the studied yeasts' ability to metabolize starch. From the biomass increments illustrated in Fig. 3 it results that *C. tropicalis* and *C. mogii* gave the highest biomass yield in starch media with pH ranging from 4.5 to 5.5. The effect of growth stimulators on the amylolytic ability of the studied



yeast extr. concentr. % w/v	1		2	
	μ(h⁻¹)	T(h)	μ(h⁻¹)	T
0.00	0.079	8.77	0.093	7.45
0.10	0.214	3.24	0.460	1.50

Fig. 4. Effect of yeast extract concentration on biomass production by *C. tropicalis* (1) and *C. mogii* (2) in Mo medium with 1% starch

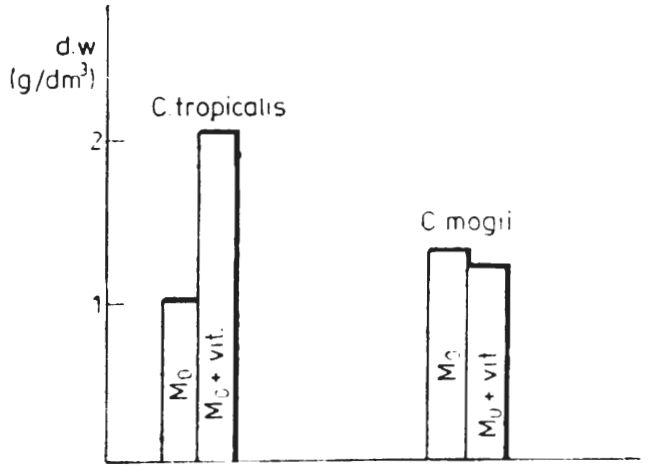


Fig. 5. Effect of biotin and thiamine on biomass production by *C. tropicalis* and *C. mogii* in Mo medium with 1% starch

yeasts is presented in Figs 4 and 5. In the standard medium containing 1% starch and 0.01-0.1% w/v of yeast extract components there was a strong stimulation of growth of the studied yeasts. With the increase of yeast extract content in the culture medium from 0 to 0.1% w/v the biomass yield increased from 0.9 to 3.0 g/dm³. Thus, a 0.1% w/v addition of this component to the Mo medium caused a threefold increase of yeast biomass with yield coefficients Y_p equal 0.33 for *C. tropicalis* and 0.35 for *C. mogii*.

The obtained results show that the yeast extract as a source of vitamins and amino acids ensured favourable development conditions for the investigated yeasts: the specific growth rate μ increased, the period of generation T was reduced, and the biomass yield increased several times (Fig. 4). The presence of thiamine and biotin in the culture medium

stimulated the growth of *C. tropicalis* only (biomass increase was 67%). The vitamins had no effect on the dynamics of growth and on biomass level in *C. mogii* cultures, this indicating the prototrophic character of these yeasts (Fig. 5). Growth of *Candida tropicalis* and *Candida mogii* in potato medium.

Table 4. Growth of *Candida tropicalis* and *Candida mogii* in potato pulp medium

Determination	*Candida tropicalis*	*Candida mogii*
CFU/cm^3	$6.33 \cdot 10^8$	$2.95 \cdot 10^8$
Nitrogen content in the sample (%)	0.140	0.126
Protein content in the samples (%)	0.875	0.788
Δ protein (%)	0.660	0.568
Starch (%)	3.14	3.14
Starch utilization (%)	66.2	43.6
$Y_b = \frac{\Delta \text{ starch}}{\text{initial starch}}$	0.21	0.18

The control of the strains' growth involved determinations of cell growth (CFU/cm^3), starch utilization, and amount of produced protein. The results are given in Table 4. The yeast count in the potato medium reached 10^8 CFU/cm^3, which amounts to a 100-fold increase of cell number in the culture medium as compared to the initial cell count. Starch utilization was from 43.6 to 66.2% and protein content in 1 dm^3 of the medium ranged from 5.68 to 6.6 g. The values of coefficient Y_b characterizing the ability to convert starch into protein, were in the range 0.18-0.21. These results show that the studied yeasts enriched the potato medium in protein; this medium has an unfavourable protein-to-carbohydrates ratio (1:8) [6]. Considering the fact that in industrial strains the biomass yield, as compared to the carbohydrates contained in the medium, is about 55% and the protein yield is 33% [1], it is clear that the isolated varieties need to have their amylolytic potential improved before becoming fit for application in biotechnology.

CONCLUSIONS

1. In the investigated starch samples there occurred yeast strains of the genera *Saccharomyces*, *Candida*, *Torulopsis*, *Trichosporon* and *Rhodotorula* (Table 2).

2. The greatest ability to metabolize starch, leading to biomass increments of 2.26-3.24 g/dm^3, was displayed by strains identified as *Candida tropicalis* and *Candida mogii* (Table 3).

3. The studied yeasts' ability to metabolize starch is a function of starch concentration, pH of the culture medium, and the presence of growth stimulators (yeast extract, thiamine and biotin) in the medium (Figs 2-5).

4. In the potato medium the strains *Candida tropicalis* and *Candida mogii* produced around 10^8 CFU/cm^3 of cells, giving a 5.68-6.60 g protein content in 1 dm^3 of medium. Starch utilization was 43.6-66.2% and the coefficient of protein yield was 0.18 and 0.21, respectively.

LITERATURA

1. Azoulay E., Jouannean F., Bertrand J. C.: Appl. Environ. Microbiol., 1980, 39. 41.
2. Augustin J., Zemek J., Kockova-Kratochvilova A., Kuniak L.: Folia Microbiol., 1978, 23, 5, 353.
3. Barnett J. A., Pankhurts R. J.: A new key to the yeast. North-Holland Publ. Co., Amsterdam and London, 1974.
4. Ebertova M.: Folia Microbiol., 1963, 8, 333.
5. Ebertova M.: Folia Microbiol., 1966, 11, 14.
6. Horubała A.: Przem. Spoż., 1978, 11, 405.
7. Janicki J., Stawicki St., Szebiotko K.: Przem. Spoż., 1968, 23, 3, 85.
8. Kotełko K., Sedlaczek L., Lachowicz T. M.: Biologia bakterii, PWN, Warszawa 1979.
9. Kockova-Kratochvilova A.: Folia Microbiol., 1982, 27, 104.
10. Lodder J.: The Yeasts, Amsterdam, London, North-Holland, 1970.
11. Maulin G., Galzy P.: Folia Microbiol., 1978, 23, 423.
12. Nakase T.: Proc IV-th Intern. Ferm. Symp. Fermen. Technol. Today G. Ferm. (ed) Kyoto, Japan 1972.
13. Spencer-Martins I., N. van Uden: Eur. J. Appl. Microb., 1977, 4, 29.
14. Sadova A. J., Veselov I. J., Gracheva I. M.: Mikrobiołogija 1968, 37, 425.
15. Sadova A. J., Veselov I. J., Gracheva I. M.: Mikrobiołogija 1969, 38, 579.
16. Struszyński M.: Analiza ilościowa i techniczna, III, Warszawa 1950.

Manuscript received: April, 1985
Authors address: 90-924 Łódź, Stefanowskiego 4/10

H. Oberman, H. Stobińska

SELEKCJA DROŻDŻY ZE ŚRODOWISK KROCHMALNICZYCH I OCENA ICH WZROSTU W ŚRODOWISKACH SKROBIOWYCH

Instytut Technologii Fermentacji i Mikrobiologii, Politechnika, Łódź

Streszczenie

W analizowanych próbach krochmalu szlamowego i rafinowanego oraz w składowanej mączce ziemniaczanej występujące szczepy drożdży reprezentowane były przez gatunki należące do rodzajów: *Saccharomyces, Candida, Torulopsis, Trichospo-*

ron i *Rhodotorula* (tab. 2). Do badań selekcyjnych wytypowano 5 szczepów drożdży rodzaju *Candida*, których zdolności wykorzystania skrobi oceniano w hodowli płytkowej i w hodowli wstrząsanej w podłożu standardowym Mo. Stwierdzono, że szczepy te miały zróżnicowane zdolności metabolizowania skrobi. Najwyższą aktywność wśród badanych drożdży wykazały szczepy zidentyfikowane jako *Candida tropicalis* i *Candida mogii* (tab. 3). Zdolności hydrolizowania skrobi u tych drożdży były funkcją stężenia skrobi, wartości pH i obecności stymulatorów wzrostu.

Najkorzystniejsze parametry wzrostu osiągały szczepy *C. tropicalis* i *C. mogii* w podłożu Mo w obecności 1% skrobi, przy wartości pH: 4,5-5,5 oraz w obecności 0,1% w/v ekstraktu drożdżowego (rys. 2, 3, 4).

Obecność tiaminy i biotyny w podłożu Mo stymulowała tylko wzrost *C. tropicalis*. Natomiast drożdże *C. mogii* wykazywały prototroficzny charakter (rys. 5). W środowisku naturalnym (podłoże ziemniaczane) badane drożdże wytworzyły liczbę komórek na poziomie 10^8 CFU/cm^3, skrobię wykorzystały w ilości od 43,6-66,2% przy wydajności białka od 18-21% (tab. 4).