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ROLE OF *P*-AMINOBENZOIC ACID (PABA) IN MODELING SELECTED PROPERTIES OF BAKERY YEAST

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Background. PABA is a growth factor; however, some papers report on the inhibiting effect of its high doses on the growth of yeast. The aim of this work was to examine the influence of PABA on growth of yeast, biomass yield, nitrogen and protein content and yeast cell morphology during cultivation on mineral and molasses media.
Material and methods. Cultures of bakery yeast *Saccharomyces cerevisiae* 2200 were run for 24 h at 28°C on a shaker at 200 rpm on mineral and molasses culture media containing 0.02, 1, 5, 10, 25, 50, 100, and 200 μg PABA in 1 cm^3.
Results. The 200 μg dose of PABA in 1 cm^3 of mineral medium resulted in the strongest growth inhibition of yeast and the lowest biomass yield. PABA addition in the molasses medium did not change the growth dynamics of the examined strain.
Conclusions. At high doses, PABA functioned as a compound that inhibited and altered the yeast growth in the mineral medium. PABA doses ranging from 0.02 to 100 μg PABA·cm^{-3} were found to evoke an increase in the nitrogen content of the cellular biomass. Upon the addition of PABA, yeast cultured in the mineral medium demonstrated a tendency to increase sizes, whereas those cultured in the molasses medium to decrease their sizes.

Key words: *Saccharomyces cerevisiae*, *p*-aminobenzoic acid, PABA, growth inhibitor

INTRODUCTION

Saccharomyces cerevisiae have found applications in all branches of the fermentation industry. For technological purposes, use is made of a population of diploid cells since, compared with haploid cells, they are characterised by faster and more active metabolism, larger sizes and, most of all, they are genetically more stable [Walker 1998]. In the proliferation of yeast, of key significance is the composition of the culture

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medium that meets basic requirements of cells, particularly the energetic cells associated with cell biosynthesis and respiration. One of the growth factors for yeast and other microorganisms, including pathogenic ones, is *p*-aminobenzoic acid.

p-Aminobenzoic acid (PABA) is an aromatic compound that is sometimes referred to vitamin H_1, B_x, or B_{10} [Chang and Hu 1996]. In cells of bacteria, yeast, fungi, and plants, PABA is synthesized from chorismate originating from the pathway of shikimic acid [Sahr et al. 2006]. Yeasts of the genus *Saccharomyces* are capable of synthesizing PABA, and the process of synthesis occurs in the cytosol [Cherest et al. 2000]. In 1993 Edman et al. reported the sequence of the gene responsible for PABA synthase at *S. cerevisiae* and designated it as gene *ABZ1* [Edman et al. 1993]. The 3840 bp DNA sequence fragment contains a 2199 bp open reading frame encoding a 733 amino acid protein with similarity to the two components of PABA synthases described for *E. coli* (PabA and PabB), suggesting that PABA synthase is bifunctional in yeast. Baker yeast contains 5-6 ppm and brewer yeast 10-100 ppm of PABA [Vasilieva 2001]. PABA is utilized by the cells in the pathway of folate synthesis and, apart from pteridine derivative and glutamic acid, constitutes the structure of a molecule of folic acid and its derivatives [Bayly and Macreadie 2002].

p-Aminobenzoic acid is a growth factor; however, some papers report on the inhibiting effect of its high doses on the growth of yeast [Reed et al. 1959, Surovtseva 1969]. The mechanism of that phenomenon has not been recognised yet. Very few references that are available failed to provide an elaborate and in-depth analysis of the abovementioned issue, whereas excessive addition of such a significant metabolite to a culture medium may trigger changes both in the morphology of cells and – what is more important – in metabolism. Reed and Surovtseva did not investigate the effect of PABA on the traits of yeast other than growth dynamics. However, they demonstrated that the culture of yeast on media containing PABA secreted a cyclic compound, identified as shikimic acid [Surovtseva 1970]. It serves as a precursor for the synthesis of antiviral agents: influenza neuraminidase inhibitor (Tamiflu ®) [Karpf and Trussardi 2001] and anticarcinogenic ones: (-)-zeylenone [Zhang et al. 2006].

It seems advisable, therefore, to extend knowledge to the effect of p-aminobenzoic acid on other traits of yeast. The aim of this work was to examine influence of PABA on growth of yeast, biomass yield, nitrogen and protein content and yeast cell morphology during cultivation on mineral and molasses media. Taking into account metabolic transformation mediated by PABA, its excess is likely to lead to the over-production of valuable components. This paper contains a part of our fundamental research data concerning the role of *p*-aminobenzoic acid in *S. cerevisiae* metabolism.

MATERIALS AND METHODS

Experiments were conducted with a diploid strain of bakery yeast *Saccharomyces cerevisiae* 2200 with identified technological properties, originating from the Pure Cultures Collection of the Department of Biotechnology and Microbiology, Warsaw University of Life Sciences.

Culture media used in the study were as follows: strictly defined mineral culture medium (A) containing mineral salts, vitamins, and 2% of glucose as a source of carbon [Verduyn et al. 1992], and molasses culture medium (B). The molasses culture was

prepared by diluting beetroot molasses with distilled water. The density was reduced from 80°Blg to 10°Blg and the mineral salts ($NH_4H_2PO_4$ – 0.19%, $(NH_4)_2SO_4$ – 0.89%) were added. A concentrated solution of PABA, sterilized through a microbiological filter Ministar (0.2 μm), was added to the culture media at dose such that the concentrations were 0.02, 1, 5, 10, 25, 50, 100, and 200 μg in 1 cm^3 of the medium. Culture media A and B without PABA addition served as controls.

The control and experimental media (A and B) were inoculated with 24-h inoculum obtained from, respectively, media A and B (without PABA addition) at a dose of 10% v/v. Cultures were run for 24 hours at a temperature of 28°C on a reciprocating shaker (SM-30 Control E. Büchler, Germany) at 200 rpm. When the culturing was stopped, the biomass was centrifuged (4000×g) for 10 minutes in a centrifuge under cooling condition, at a temperature of 4°C (Centrifuge MPW-365, Poland).

The optical densities (OD) of the culture were measured at 0, 3, 6, 9, and 24 hour of culture by means of a spectrophotometer (Spectronic 20 Genesys, USA) at a wavelength of 600 nm. The yield of cell biomass was determined using the drying method according to AOAC guidelines (1995), using an SML 32/250 dryer manufactured by Zelmet, Poland. The results of biomass yield were presented per 1 dm^3 of medium (g d.m.·dm^{-3}).

The total nitrogen in the yeast cell biomass determined using the Kiejdahl method was carried out following AOAC standards. The sample was mineralized in a combustion apparatus (Büchi Digestion Unit K-435, Germany) and distilled in a distillation apparatus (Büchi 316 Distillation Unit, Germany). The protein content was calculated based on the results of nitrogen content multiplied by a factor of 6.25. The factor, in turn, was computed based on an average nitrogen content of proteins that accounts for 16% [AOAC 1995].

The morphology of yeast cells was evaluated in a 24-hour culture. Images of cells were taken using a light microscope (Zeiss Axiostar plus, Germany) and a digital camera (Sony DSC-S75). After the images were taken, four values were measured: length, width, circumference, and surface area of cells. For each combination, 100 measurements were made using the software, Zeiss LSM Image Browser.

RESULTS

Effect of *p*-aminobenzoic acid on growth dynamics of yeast

The course of changes in the optical density of *S.c.* 2200 culture in the mineral media (A) containing *p*-aminobenzoic acid at concentrations of 0.02, 1, 5, 10, and 25 μg·cm^{-3} was consistent with the course of changes observed in the control medium (Fig. 1). Growth inhibition of yeast in the mineral medium after a few hours of culture was noted when the content of PABA reached 50 and 100 μg·cm^{-3} medium. Still, the optical density at the final hour of the culture was similar to that recorded in the control medium. The 200 μg dose of PABA in 1 cm^3 of medium resulted in the strongest growth inhibition of yeast. It should be assumed that over the entire period of culture in the media containing 50, 100, and 200 μg PABA·cm^{-3}, the cells were in the initial phases of growth and failed to reach the stationary phase.

Optical density of *S.c.* 2200 yeast culture run on the molasses medium (B) with the addition of the analysed doses of PABA (Fig. 2) showed an increase at all the examined

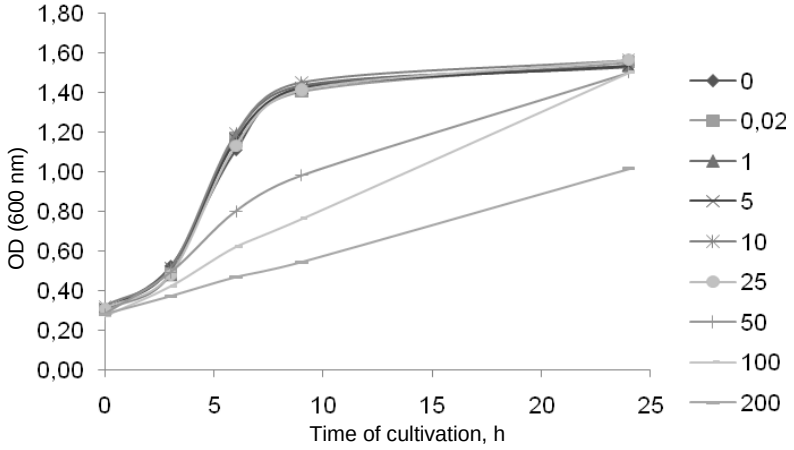

Fig. 1. The changes in the optical density (OD) of *Saccharomyces cerevisiae* 2200 yeast culture during cultivation in the mineral medium (A) with PABA addition

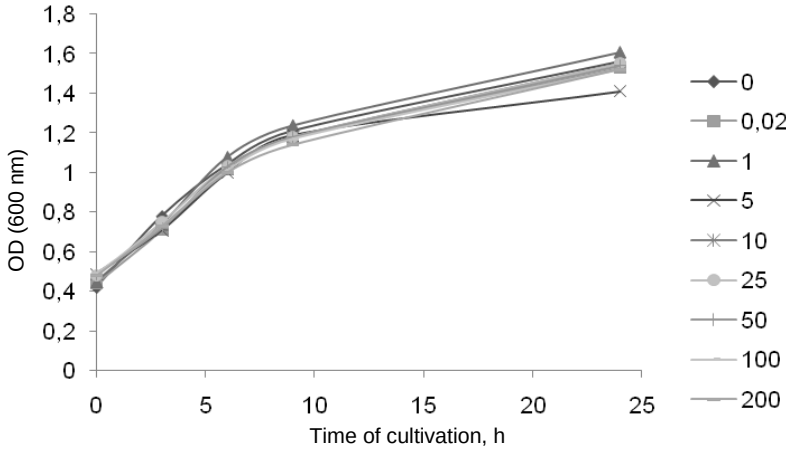

Fig. 2. The changes in the optical density (OD) of *Saccharomyces cerevisiae* 2200 yeast culture during cultivation in the molasses medium (B) with PABA addition

time intervals and its changes were very similar to those of OD reported in the control medium. When referenced to a growth curve, those changes indicate that the adaptive phase of the examined yeast might have occurred and even if it did, it was very short and the strain reached the logarithmic phase of growth rapidly. It should be concluded that PABA addition at doses ranging from 0.02 to 200 $\mu g \cdot cm^{-3}$ in the molasses medium did not change the growth dynamics of the examined strain *Saccharomyces cerevisiae*.

Effect of *p*-aminobenzoic acid on biomass yield

Capacity of cells to proliferate rapidly and efficiently, expressed as biomass yield, is one of the key criteria in the evaluation of the effect of specific factors of a culture medium on all the metabolic processes occurring in a cell.

Depending on PABA concentration in the medium, biomass yield of *S.c.* 2200 strain cultured on the medium A (Fig. 3) ranged from 2.22 to 3.24 g d.m.·dm^{-3}. The lowest biomass yield was obtained for yeast cultured on the medium containing 200 µg PABA·cm^{-3} whereas the highest one was obtained from the medium containing 0.02 µg PABA per 1 cm^3. No significant differences were found between biomass yields obtained from the control medium and those obtained from the medium containing 0.02 µg PABA·cm^{-3}. p-aminobenzoic acid applied at a dose of 1 µg·cm^{-3} of medium was found to significantly diminish the biomass yield; however, differences between biomass yields obtained from the cultures with the higher PABA doses were not significant.

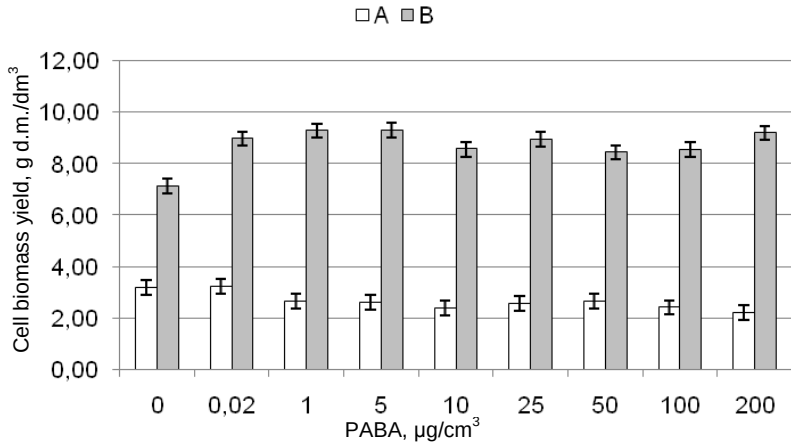

Fig. 3. The cell biomass yield of *Saccharomyces cerevisiae* 2200 yeast after 24-h cultivation in the mineral (A) and molasses medium (B) with PABA addition

Analyses of the impact of PABA content in the molasses medium B on *S.c.* 2200 yeast proliferation (Fig. 3) demonstrated that their biomass yield ranged from 7.15 to 9.32 g d.m.·dm^{-3}. The lowest yield of 2200 yeast biomass was obtained from the culture medium and the highest one was obtained from the medium supplemented with 5 µg PABA·cm^{-3} of medium. It should be emphasized that, irrespective of the PABA dose, the biomass yield of 2200 yeast cells from cultures in all experimental media was significantly higher than that recorded in the control medium.

Effect of *p*-aminobenzoic acid on the contents of total nitrogen and protein

The content of total nitrogen (Fig. 4) in cells of 2200 yeast cultured on the mineral medium A ranged from 7.59 g N·g^{-1}d.m.$^{-1}$ for PABA dose of 200 µg·cm^{-3} to 11.65 g N·g^{-1} d.m.$^{-1}$ for PABA dose of 1 µg·cm^{-3}. During culture in that medium, *p*-aminobenzoic acid

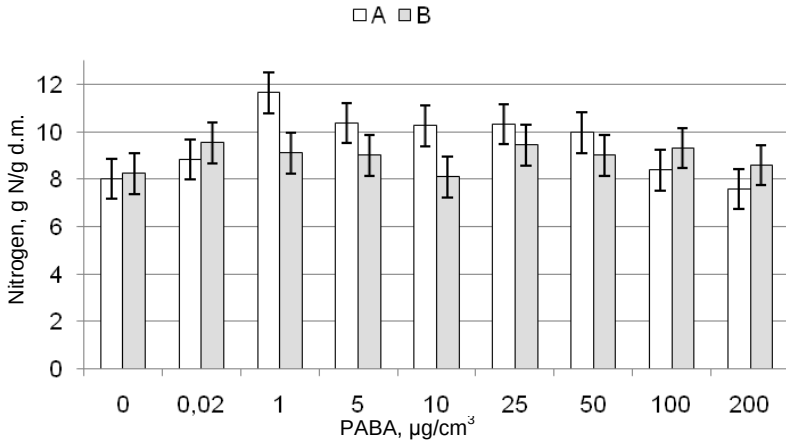

Fig. 4. The content of nitrogen in biomass of *Saccharomyces cerevisiae* 2200 yeast obtained from 24-h cultures in the mineral medium (A) and molasses medium (B) with PABA addition, g N/g d.m.

was found to increase the accumulation of nitrogen in the cells of the analysed yeast strain. Compared with the cells cultured without PABA addition, a significantly higher content of nitrogen was observed in cells cultured in media containing 1 to 50 µg PABA·cm^{-3}. The doses of 100 and 200 µg PABA·cm^{-3} appeared to significantly reduce the capacity of the cells to accumulate nitrogen, compare with doses of 1-50 µg PABA·cm^{-3}.

The nitrogen content determined in yeast biomass obtained in the molasses culture medium (B) fluctuated between 8.1 and 9.54 g N·g^{-1} d.m.$^{-1}$. The lowest examined dose of PABA, i.e. 0.02 µg PABA·cm^{-3} of medium, was found to significantly increase nitrogen content of the yeast cells. As compared to the effect evoked by the dose of 0.02 µg PABA·cm^{-3} of medium, the content of nitrogen was not significantly affected by the other doses examined.

In the case of 2200 yeast cultured on the medium A, a significantly higher content of protein (Table 1) was observed at the following doses of PABA: 1, 5, 10, 25, and 50 µg PABA·cm^{-3}. The lowest protein content corresponded to biomass obtained after the culture in the medium supplemented with 200 µg PABA·cm^{-3}. A significant increase in protein content of the biomass obtained from the medium B was noted for doses

Table 1. The content of protein in biomass of *Saccharomyces cerevisiae* 2200 yeast obtained from 24-h cultures in the mineral medium (A) and molasses medium (B) with PABA addition, g/g d.m.

PABA µg/cm^3		0	0.02	1	5	10	25	50	100	200
Protein g/g d.m.	**A**	50.13^{a}	55.31^{b}	72.81^{d}	64.88^{c}	64.13^{c}	64.50^{c}	62.31^{c}	52.38^{a,b}	47.44^{a}
	B	51.56^{a}	59.63^{b,c}	56.88^{b}	56.31^{b}	50.63^{a}	59.06^{b,c}	56.38^{b,c}	58.19^{b,c}	53.69^{b}

The same letter index denotes a lack of significant difference.

exceeding 0.02 μg PABA·cm^{-3}. It is noteworthy that, apart from the PABA dose, the type of medium determined the content of protein in the cell biomass of the investigated yeast strain.

Effect of *p*-aminobenzoic acid on cell morphology

In the analyses of the impact of PABA on the morphology of cells, the measurements of length, width, circumference, and surface area of cells were made from the images that were obtained, whereas a shape coefficient was computed as a quotient of cell length to its width (Table 2).

Table 2. The surface, circumference and shape coefficient of *Saccharomyces cerevisiae* 2200 yeast obtained from 24-h cultures in the mineral medium (A) and molasses medium (B) with PABA addition

PABA μg/cm^3	Shape coefficient length of cell/width of cell		Surface μm^2		Circumference μm	
	A	B	A	B	A	B
0	1.13	1.36	27.21^a	28.54^a	18.82^a	18.99^a
0.02	1.28	1.36	29.01^{a,c}	24.38^b	19.37^{a,c}	17.86^b
1	1.22	1.30	30.14^c	27.09^a	19.45^{a,c}	18.77^a
5	1.25	1.27	30.50^c	26.36^{a,b}	19.84^c	18.37^a
10	1.33	1.30	27.63^a	23.44^b	18.94^a	17.36^b
25	1.26	1.37	29.79^c	22.74^b	19.38^{a,c}	17.13^b
50	1.24	1.35	30.96^a	26.26^{a,b}	17.62^b	18.14^b
100	1.14	1.26	30.76^c	24.37^b	18.83^a	17.99^b
200	1.20	1.30	27.44^a	27.20^a	18.00^b	18.09^b

The same letter index denotes a lack of significant difference.

Circumference and surface area of the cells of the analyzed yeast originating from the control media A and B were similar. The addition of PABA to the media appeared to change the morphology of the cells. A general tendency for increase in the surface area of cells and their circumference was observed when PABA was added to the medium A. The largest surface area was noted for the cells obtained from a culture in the medium containing 50 μg PABA·cm^{-3}, whereas the largest circumference was obtained for those originating from the medium containing 5 μg PABA·cm^{-3}. Changes in the morphology of cells after culture in the molasses medium were different from those observed in the mineral medium since in the latter a tangible reduction was observed in cell size. PABA added to the medium B significantly decreased both the circumference and surface area of cells, which was most significant in the case of the PABA dose of 25 μg·cm^{-3}. A comparison of shape coefficients demonstrated that PABA did not affect the shape of cells. In contrast, the morphological characteristics were determined, to a significant extent, by the type of the culture medium. Irrespective of the PABA

dose applied, cells cultured in the medium B were more elongated as compared to those cultured in medium A.

DISCUSSION

The changes observed in the growth dynamics of yeast cultured in the mineral medium (A) evoked by the presence of *p*-aminobenzoic acid confirmed previous literature data on the inhibiting effect of that compound on yeast growth. According to Reed [1959], the lowest dose that impaired the growth of yeast was 25 μg PABA·cm^{-3} of medium, whereas according to Surovtseva [1969] that dose accounted for as much as 1 mg·cm^{-3}. The results obtained in this study show that the inhibition of yeast growth occurred when the content of PABA in 1 cm^3 of the mineral medium reached 50 μg. It should be emphasized that after 24 hours of culture, the inhibiting effect, indicated by a considerable decrease of optical density, could be observed only at the highest examined dose of PABA, i.e. 200 μg·cm^{-3}. Comparison of the changes in the optical density with the course of growth curves demonstrate that the application of PABA at the concentration of 50 to 200 μg·cm^{-3} of medium extended the initial growth phase of cells to a considerable extent. This was particularly visible in the phase of adaptation of the cells to environmental conditions.

No properties inhibiting the growth of cells were observed in the molasses medium (B), whereas a known dependency was confirmed between the type of culture medium and potential for development of cells. It should be assumed that the molasses medium, richer than the mineral medium in available nutrients, may contain such compounds that eliminate the unfavourable effect of *p*-aminobenzoic acid on cells of the strain examined.

Irrespective of the PABA doses applied, there are substantial differences in the yield of the cellular biomass of *S.c.* 2200 strain obtained from cultures in mineral and molasses media. It may be assumed that they result from a difference in the content of saccharides in both the culture media. Saccharide content of the molasses medium was 2.5--fold higher than that of the mineral medium, which corresponds to similar differences between cell biomass yields. The greatest differences (6.47 g d.m.·dm^{-3}) between the yields obtained in the experimental media were reported at the PABA dose of 25 μg·cm^{-3}. The results indicate that the rate of metabolic processes of 2200 strain, expressed by biomass yield, was determined to a significantly greater extent by the type of culture medium used than by the PABA doses applied.

A similar observation was also found in the analysis of results referring to changes in cell morphology evoked by various doses of PABA. The surface area and circumference of the cells were affected mainly by the type of the culture medium. In the molasses medium containing PABA, the cells were characterised by the smallest surface area and circumferences. In turn, addition of PABA to the mineral medium increased those measures.

PABA contains an amine group, hence it may be expected that under conditions of increased access to PABA, the yeast cells will be characterised by a higher content of nitrogen. In the reported study, PABA doses ranging from 0.02 to 100 μg PABA·cm^{-3} were found to evoke an increase in the nitrogen content of the cellular biomass. In contrast, the dose of 200 μg PABA·cm^{-3}, significantly inhibiting the growth of yeast, appeared to reduce the nitrogen content to a level noted in the control medium.

It is worth emphasizing that in the biomass of *S.c.* 2200 strain, the high content of total nitrogen was obtained both in the control and in the experimental cultures. In all the cases, the protein content exceeded 50% and 60% in case of cultivating on the molasses medium. Biomass of *Candida utlilis* yeast cultures on the YPG medium supplemented in salt of magnesium also was rich in protein (over 60%) [Błażejak 2006]. The content of proteins in yeast is a significant parameter affecting basic technological characteristics of yeast, namely, the propelling force and stability. The increasing protein content (in range 38-42%) causes increasing the fermentation ability [Lipińska et al. 1997, Gniewosz et al. 1997]. It is speculated that an increased content of protein (over 45%) implies increased amount of intracellular enzymes that support the process of autolysis, thus leading to a decrease in stability [Lipińska et al. 1997, Sobczak et al. 1997]. Simultaneously, when protein content exceeds 50%, the fermentation activity, expressed by the propelling force, increases. Thus, based on literature data, it may be expected that biomass obtained under the described culture conditions will be characterized by a high propelling force and low stability. Values of those technological parameters should depend on the content of PABA in the medium as well as on the type of medium. It seems that particularly the dose of 200 μg PABA·cm^{-3}, which inhibited the growth of cells and diminished biomass yield, is likely to change technological properties of yeast. In the future, those speculations should, however, be supported with experimental data.

CONCLUSIONS

1. At high doses, PABA functioned as a compound that inhibited and altered the yeast growth in the mineral medium. The application of PABA at the concentration of 50 to 200 μg·cm^{-3} extended the initial growth phase of cells.

2. No properties inhibiting the growth of cells of the strain *S. cerevisiae* 2200 were observed in the molasses medium which may contain compounds that eliminate the unfavourable effect of *p*-aminobenzoic acid. It was also not found the dose of PABA reducing the biomass yield in the molasses medium

3. PABA doses ranging from 0.02 to 100 μg PABA·cm^{-3} were found to evoke an increase in the nitrogen content of the cellular biomass

4. The rate of metabolic processes of 2200 strain, expressed by biomass yield, was determined to a significantly greater extent by the type of culture medium used than by the PABA doses applied.

5. Upon the addition of PABA, yeast cultured in the mineral medium demonstrated a tendency to increase sizes, whereas those cultured in the molasses medium to decrease their sizes.

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ROLA KWASU *P*-AMINOBENZOESOWEGO (PABA) W MODELOWANIU WYBRANYCH WŁAŚCIWOŚCI DROŻDŻY PIEKARSKICH

Wprowadzenie. PABA jest znany jako czynnik wzrostu, jednakże niektóre publikacje donoszą, że wpływa on hamująco na wzrost drożdży. Celem pracy było sprawdzenie

wpływu PABA na dynamikę wzrostu drożdży, plon biomasy, zawartość azotu i białka oraz na morfologię komórek drożdżowych hodowanych w podłożu mineralnym i melasowym.
Materiały i metody. Drożdże *Saccharomyces cerevisiae* 2200 hodowano przez 24 h w 28°C na wytrząsarce (200 rpm) w podłożu mineralnym i melasowym zawierającym 0,02, 1, 5, 10, 25, 50, 100, oraz 200 μg PABA w 1 cm^3.
Wyniki. Dawka 200 μg PABA w 1 cm^3 podłoża mineralnego wykazała największe właściwości hamujące wzrost drożdży oraz powodowała najniższy plon biomasy. W podłożu melasowym duże dawki PABA nie wpływały na zmiany przebiegu OD.
Wnioski. Duże dawki PABA dodawane do podłoża mineralnego działały jako inhibitor wzrostu drożdży. Dodatek PABA do podłoża melasowego nie zmieniał dynamiki wzrostu badanego szczepu drożdży i nie wpływał na plon biomasy. PABA w dawkach od 0,02 do 100 $\mu g \cdot cm^{-3}$ wpływał na zwiększenie zawartości azotu w biomasie komórkowej. Zaobserwowano, iż rodzaj stosowanego podłoża ma istotne znaczenie dla morfologii komórek. Podczas hodowli w podłożu mineralnym z PABA drożdże wykazywały tendencję do zwiększania rozmiarów, natomiast w hodowli w podłożu melasowym do ich zmniejszania w porównaniu z próbą kontrolną.

Słowa kluczowe: *Saccharomyces cerevisiae*, kwas *p*-aminobenzoesowy, PABA, inhibitor wzrostu

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