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THE GROWTH OF *SACCHAROMYCES CEREVISIAE* YEAST IN CADMIUM ENRICHED MEDIA

Anna Pasternakiewicz
Rzeszów University

Abstract. The effect of Cd^{2+} ions on the growth of *Saccharomyces cerevisiae* yeasts grown in a natural and a synthetic medium was studied. Two strains, B_1 i G_1, were used. Both strains displayed high sensitivity to increased cadmium salt contents, particularly those grown in a synthetic medium. High concentrations of cadmium (> 50 μM) practically stopped the growth of the studied strains. The negative effect of cadmium ions was partly altered by adding zinc or calcium salts to the synthetic YM medium.

Key words: *Saccharomyces cerevisiae*, growth, metal ions, interactions

INTRODUCTION

Metal ions play an important role in the life processes of organisms. Depending on the kind of organism, its age, physiological developmental phase, habitat conditions (such as temperature or pH), the physiochemical properties of the metal, co-occurrence of other metal ions and chemical compounds, both the required and the toxic contents of metals may vary.

For many elements we can determine the lowest concentrations, below which there occurs a deficit, and the highest tolerated levels, above which the element has a negative effect on the life processes of an organism, in even higher concentrations causing death [Kaim and Schwederski 1994].

Cadmium is an element, which is highly toxic to living organisms. The potential danger of contaminating the environment with cadmium is large, as the element undergoes anthropogenic concentration and easy bioaccumulation. The largest contaminating agents are factories producing zinc, nickel and other non-Fe metals. It is estimated that over half of the global production of cadmium comes from the metal industry. The cadmium content of soils is often very high, especially in industrial areas. From soils it can easily penetrate to the surface and to underground waters. Cadmium toxicity in waters is high, and its absorption both by water plants and animals is proportional to its concentration in water [Kabata-Pendias and Pendias 1993].

Corresponding author – Adres do korespondencji: Dr Anna Pasternakiewicz, Department of General and Physiological Chemistry of Rzeszów University, Ćwiklińskiej 2, 35-601 Rzeszów, Poland, e-mail: apast@univ.rzeszow.pl

MATERIALS AND METHODS

Two strains, B_1 and G_1, of *Saccharomyces cerevisiae* yeast were used in the study. These strains were created by the protoplast fusion method and can be characterized by a high substrate efficiency and high productivity [Sałek 1989]. The reproduction was carried out in two media – a natural one, i. e. brewery wort, and the synthetic YM yeast medium [Burbianka and Pliszka 1977]. The media were enriched with cadmium (II) sulphate (VI), in such quantities that initial Cd^{2+} concentrations were: 1, 10, 50, 100 and 200 μM.

The source of metal ions in the experiment with pairs of ions were the following salts: $CaCl_2$, $ZnSO_4$, $MnCl_2$, $Pb(NO_3)_2$ and $CuSO_4$.

Sterile medium (10 cm^3) was placed in conical flasks, inoculated with pure yeast culture and left in an incubator (30°C) for 24 hours. The suspension was then moved to conical flasks of 100 cm^3 of the medium with cadmium added in set proportions. Then the dynamic culture was carried for 48 hours (amplitude 3, 180 cycles/min, 30°C). During the process the growth of yeast was controlled by measuring the absorbance of the culture. After completion of the yeast production the suspension was centrifuged (3000 × g, 10 min) and three times washed with redistilled water. The wet biomass was dried for 2 hours at 60°C, and then at 105°C until it was a constant weight. On the basis of dry weight, the kinetic parameters of the process were obtained, such as the proper growth speed and time of biomass doubling [Miśkiewicz and Leśniak 1975].

Then the data were statistically tested. The effect of cadmium concentration in the medium on yeast growth was estimated using Two-way Analysis of Variance (ANOVA), which was calculated with Statistica computer package, version 5'1997.

RESULTS AND DISCUSSION

The experiment showed the negative influence of cadmium ions on the growth of *Saccharomyces cerevisiae* yeast. The studied strains were more resistant to the increased presence of Cd^{2+} in brewery wort than in the synthetic medium. The differences in tolerance must arise from the differing chemical compositions. Large molecules, present in brewery wort might have bound some of the cadmium ions, creating insoluble compounds or large complexes, not able to penetrate through cell membranes into cell interiors.

The shape of the absorbance curve during the growth of cultures indicates the inhibition of yeast cell reproduction (Fig. 1). The curves, both for B_1 and G_1 strains, were higher than the control one only for the 1 μM cadmium concentration in the natural medium. With increasing Cd^{2+} content the curves were flatter and flatter. Also in the synthetic medium the absorbance gradually decreased with higher concentrations of cadmium. In the conditions present in this experiment, both for natural and synthetic medium, the concentration of 200 μM Cd^{2+} caused the total inhibition of yeast growth.

Cadmium ions introduced in the natural medium with the concentration of 1 μM hardly influenced the growth of the studied strains (Table 1). For higher concentrations a gradual decrease of the produced biomass was observed. For 50 μM Cd^{2+}, the produced dry weight decreased by 0.093 g, and for 200 μM – by 0.590 g. The speed of growth lowered respectively as well as the length of time needed for biomass doubling.

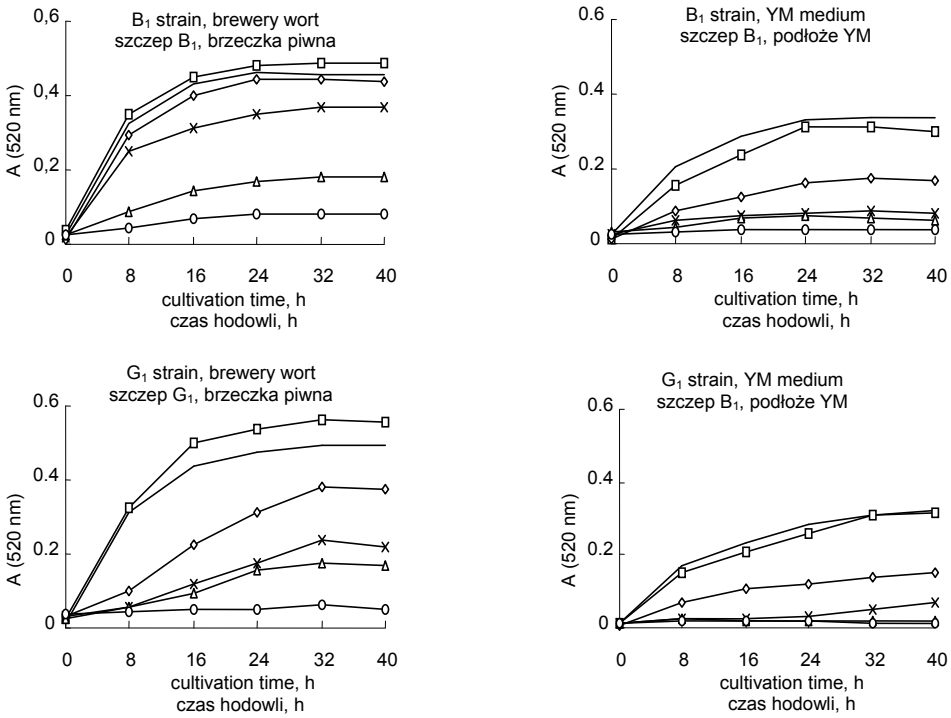

Fig. 1. Medium absorbance changes in the *Saccharomyces cerevisiae* yeast culture with various of cadmium salts: —— control, —□— 1, —◊— 10, —×— 50, —Δ— 100, —○— 200 μM
Rys. 1. Zmiany absorbancji podłoża podczas hodowli drożdży *Saccharomyces cerevisiae* przy zwiększonej zawartości soli kadmu: —— kontrola, —□— 1, —◊— 10, —×— 50, —Δ— 100, —○— 200 μM

Table 1. Influence of Cd^{2+} ions on the biomass production (DM), specific growth rate (μ) and time of biomas doubling (T) – brewery wort
Tabela 1. Wpływ jonów Cd^{2+} na plon biomasy drożdży (DM), właściwą szybkość wzrostu (μ) i czas podwojenia biomasy (T) – podłoże naturalne

Content of Cd^{2+} in the medium, μM Zawartość Cd^{2+} w podłożu, μM	B_1 strain – Szczep B_1			G_1 strain – Szczep G_1		
	DM ± SD, g	μ, h^{-1}	T, h	DM ± SD, g	μ, h^{-1}	T, h
Control – Kontrola	0.958± 0.018 a	0.161	4.30	1.070± 0.042 A	0.134	5.17
1	1.036± 0.032 a	0.133	5.21	1.038 ± 0.036 A	0.133	5.21
10	0.942± 0.017 a	0.131	5.29	0.811± 0.010 B	0.127	5.46
50	0.865± 0.036 b	0.130	5.33	0.497± 0.050 C	0.115	6.03
100	0.483± 0.028 c	0.114	6.08	0.452± 0.044 C	0.113	6.13
200	0.368± 0.041 d	0.107	6.48	0.223± 0.040 C	0.095	7.29

SD – standard deviation, a, b, c..., A, B, C... – means with the same superscripts letters are not significantly different.

SD – odchylenie standardowe, a, b, c..., A, B, C... – te same litery oznaczają brak statystycznie istotnej różnicy.

The biomass yield for the synthetic YM medium was, for both B_1 and G_1strains, lower in the medium enriched with cadmium. Although for 1 μM concentration of $CdSO_4$, the dry weight of yeast was similar to the control part, higher concentrations of cadmium drastically decreased it (Table 2). The 10 μM concentration of Cd^{2+} lowered the biomass yield for G_1 strain by ca. 60%, whereas higher concentrations lowered it by 75-80% respectively. The speed of growth lowered in the same fashion, as well as the length of time needed for biomass doubling, which was 2.5 hours longer than the control. The Two-way Analysis of Variance showed that cadmium significantly reduced the biomass yield of yeasts.

Table 2. Influence of Cd^{2+} ions on the biomass production (DM), specific growth rate (μ) and time of biomas doubling (T) – YM medium
Tabela 2. Wpływ jonów Cd^{2+} na plon biomasy drożdży (DM), właściwą szybkość wzrostu (μ) i czas podwojenia biomasy (T) – podłoże YM

Content of Cd^{2+} in the medium, μM Zawartość Cd^{2+} w podłożu, μM	B_1 strain – Szczep B_1			G_1 strain – Szczep G_1		
	DM ± SD, g	μ, h^{-1}	T, h	DM ± SD, g	μ, h^{-1}	T, h
Control – Kontrola	0.575± 0.088 a	0.140	4.95	0.608± 0.058 A	0.120	5.78
1	0.542± 0.041 a	0.117	5.92	0.567± 0.054 B	0.118	5.87
10	0.323± 0.031b	0.105	6.60	0.347± 0.017 C	0.106	6.54
50	0.184± 0.009 c	0.090	7.68	0.155± 0.010 D	0.086	8.06
100	0.142± 0.017 d	0.084	8.23	0.150± 0.024 D	0.085	8.15
200	0.137± 0.022 d	0.083	8.84	0.131± 0.022 D	0.081	8.56

SD – standard deviation, a, b, c..., A, B, C... – means with the same superscripts letters are not significantly different.
SD – odchylenie standardowe, a, b, c..., A, B, C... – te same litery oznaczają brak statystycznie istotnej różnicy.

The achieved results agree with the results of Jones and Greenfield [1984], who found that the growth of *S. cerevisiae* is slowed by concentrations of cadmium higher than 10 μM, and entirely stops at around 1 mM. It can be presumed that a high level of cadmium in the medium impairs the synthesis of proteins in yeast cells. Cadmium can also cause structural damage in the plasma of the membranes of cell walls due to binding with organic ligands present in it [Brady and Duncan 1994]. The absorption of cadmium is accompanied by freeing of intracellular potassium, a biologically important element. It constitutes a centre of regulation of bivalent ions and in its presence the toxic influence of other ions – inhibitors, present in the cytoplasm, decreases [Norris and Kelly 1977]. Low concentrations of cadmium in cells did not cause significant aberrations in the functioning of reproducing *Saccharomyces cerevisiae* cells, and even could have stimulated the occurrence of some protective mechanisms. These mechanisms are based on the synthesis of intracellular proteins containing cystein. Cystein can bind heavy metals creating chelates. Chelates have no toxic impact on cells of microorganisms and cause the decrease of *de facto* concentrations of metal ions in the medium [Brady et al. 1994].

Bioaccumulation of heavy metals takes place through two processes: by active or passive absorption into the interior of cells, or by biosorption through the cell wall. Cell

walls have a negative charge, caused by the dissociation of some chemical groups (eg. carboxylic, hydroxylic and phosphatic), hence they can bind metals using ionic or co-ordinative bonds. Negatively charged groups in the cell wall can to a large extent decide the kinetics of absorption of other charged groups (eg. metal ions) on the cell surface [Chen and Ting 1995]. Heavy metals, including cadmium, belong to the so called Pearson's soft acids, which have a tendency to bind with soft polarized ligands, including those containing nitrogen and sulphur. There are more such soft bond places in the interior of the cell than in the cell wall, which hardly constitutes a barrier against heavy metals [Brady and Duncan 1994, Lippard and Berg 1998].

Avery and Tobin [1993] showed in their study that the bioaccumulation of cadmium in *Saccharomyces cerevisiae* yeast cells takes place very quickly – after five minutes of incubation in a culture containing 50 μM Cd^{2+}, the level of cadmium in the cells was 0.2 μM/g of dry weight. Similarly, Volesky and May-Philips [1995] detected 0.16 μM of cadmium in 1 g of biomass after incubation in a 20 μM $Cd(NO_3)_2$ solution. Absorption of cadmium into the cells is related to its concentration in the medium [Blaudez et al. 2000]. Thus, it can be assumed that in the studied cultures cadmium compounds quickly penetrated into the cells' cytoplasm from the media and inhibited life processes.

The unfavourable effect of cadmium salts (100 μM) on the growth of the G_1 strain was partly altered by adding a zinc compound (100 μM) to brewery wort. Then the biomass yield was 0.481 g, in contrast to 0.452 g with Cd^{2+} only. An even better result was gained after adding calcium salts to the medium (100 mM). Then the biomass yield was ca. 0.09 g higher than when only Cd^{2+} was added. Similar results were obtained by Norris and Kelly [1997] – an addition of 0.2 mM $CaSO_4$ to a culture with 0.2 mM $CdSO_4$ caused a considerable decrease of the bioaccumulation of cadmium in cells.

The addition of manganese to the natural medium with the G_1 yeast strain did not alter the negative influence of cadmium (Fig. 2).

The introduction of calcium and zinc ions in the natural medium with the B_1 yeast strain culture altered the negative influence of cadmium to a lesser extent than in the case of the other strain. The presence of lead, which is, similarly to cadmium, a heavy metal, markedly lowered the biomass yield. Thus these elements have additive negative influence.

Supplementing the YM medium with calcium or zinc compounds, which positively influence the growth of yeast, partly altered the inhibiting effect of cadmium. The dry weight in this part of the experiment was higher than for cultures grown in beer wort with cadmium but without zinc or calcium, but still much lower than in the control part (without cadmium).

The addition of 100 μM Pb^{2+} salts to brewery wort increased the dry biomass yield by 10% for the B_1 strain, and by 2% for the G_1 strain, compared to the control part. However, in the synthetic medium the results were reversed. The same addition of Pb^{2+} lowered the dry weight yield by 7-8% compared to the control [Pasternakiewicz 2006]. Biomass yields for the B_1 and G_1 strains in the media enriched both with Pb^{2+} and Cd^{2+} were lower than in the case of media enriched only with one of these elements.

The presence of 100 μM $CuSO_4$ in both natural and synthetic media lowered biomass yields for B_1 and G_1 yeast strains. Simultaneous introduction of Cd^{2+} and Cu^{2+} gave yields which were only slightly lower compared to the sole addition of cadmium (Fig. 2).

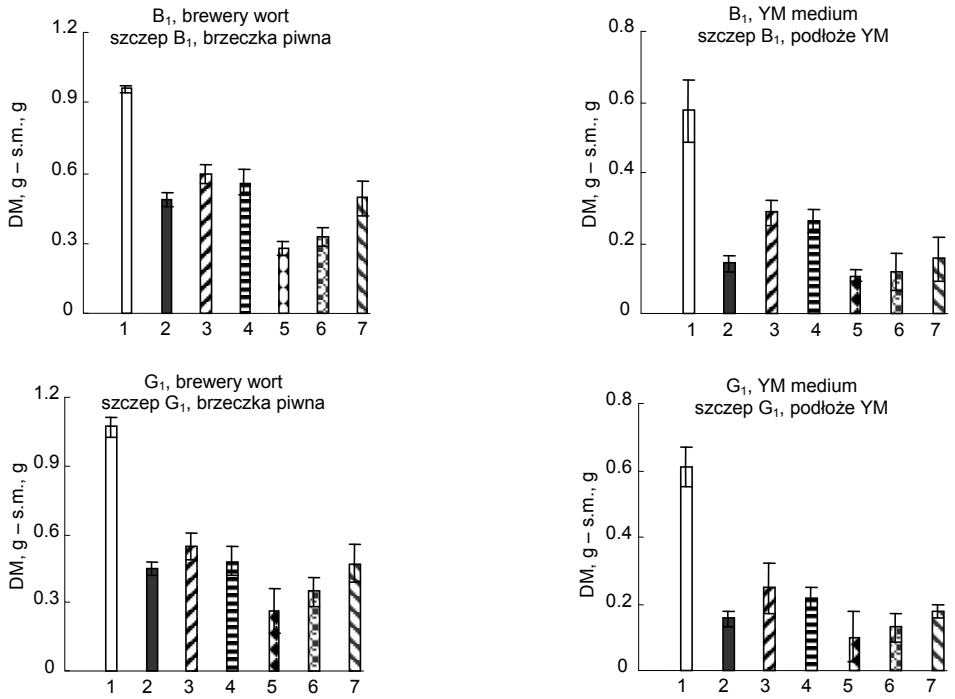

Fig. 2. Influence of the salts of chosen metals on the *Saccharomyces cerevisiae* dry mass production in media with increased cadmium content: 1 – control, 2 – 100 μM Cd^{2+}, 3 – 100 μM Cd^{2+} + 100 mM Ca^{2+}, 4 – 100 μM Cd^{2+} + 100 μM Zn^{2+}, 5 – 100 μM Cd^{2+} + 100 μM Pb^{2+}, 6 – 100 μM Cd^{2+} + 100 μM Cu^{2+}, 7 – 100 μM Cd^{2+} + 100 μM Mn^{2+}

Rys. 2. Wpływ soli wybranych metali na plon suchej masy (s.m.) szczepów *Saccharomyces cerevisiae* w obecności soli kadmu w pożywce: 1 – kontrola, 2 – 100 μM Cd^{2+}, 3 – 100 μM Cd^{2+} + 100 mM Ca^{2+}, 4 – 100 μM Cd^{2+} + 100 μM Zn^{2+}, 5 – 100 μM Cd^{2+} + 100 μM Pb^{2+}, 6 – 100 μM Cd^{2+} + 100 μM Cu^{2+}, 7 – 100 μM Cd^{2+} + 100 μM Mn^{2+}

The favourable effect of zinc ions in reversing the inhibiting effect of cadmium was also confirmed by Tomasik and Warren [1996].

Metals which are toxic to microorganisms can enter their cells by the use of "selective" transporters. Cu^{2+} ions can play such a role for cobalt [Thomine et al. 2000]. Whereas the introduction of calcium or magnesium salts, which are Pearson's hard acids, favoured the creation of large complex molecules containing these elements and parts of the cell wall. These large molecules then blocked the places of cadmium entry into the cytoplasm and in this way lowered cadmium's negative influence on yeast growth.

SUMMARY – CONCLUSIONS

1. The presence of Cd^{2+} in yeast media, in concentrations above 10 μM, had a negative effect on the growth of *Saccharomyces cerevisiae* yeasts.

2. Yeasts grown in brewery wort, which is a natural medium with rich quantitative and qualitative composition, had a higher tolerance to the presence of cadmium, compared to the yeasts grown in the YM synthetic medium.

3. The negative effect of higher concentrations of cadmium was slightly lowered by the addition of calcium or zinc salts.

4. The growth inhibiting effect of Cd^{2+}, Pb^{2+} and Cu^{2+} was increased when Cd^{2+} – Pb^{2+} or Cd^{2+} – Cu^{2+} pairs were applied in the medium instead of one of these elements.

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WZROST DROŻDŻY *SACCHAROMYCES CEREVISIAE* PRZY ZWIĘKSZONEJ ZAWARTOŚCI ZWIĄZKÓW KADMU W PODŁOŻU

Streszczenie. Badano wpływ jonów Cd^{2+} na wzrost drożdży *Saccharomyces cerevisiae*, szczepu B_1 i G_1, namnażanych na podłożu naturalnym i syntetycznym. Obydwa stosowane w doświadczeniach szczepy drożdży wykazywały dużą wrażliwość na obecność zwiększonych stężeń soli kadmu w pożywce, szczególnie hodowane na podłożu syntetycznym. Duże zawartości kadmu w podłożu (> 50 μM) przyczyniały się do niemal całkowitego zahamowania wzrostu badanych szczepów. Niekorzystne działanie jonów kadmu było częściowo zniesione po wprowadzeniu do syntetycznego podłoża YM soli cynku lub wapnia.

Słowa kluczowe: *Saccharomyces cerevisiae*, wzrost, jony metali, interakcje

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