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REGULAR ARTICLE

PREPARATION OF ZINC ENRICHED YEAST (*SACCHAROMYCES CEREVISIAE*) BY CULTIVATION WITH DIFFERENT ZINC SALTS

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ABSTRACT

The yeast *Saccharomyces cerevisiae* is the best known microorganism and therefore widely used in many branches of industry. This study aims to investigate the accumulation of three inorganic zinc salts. Our research presents the ability of this yeast to absorb zinc from liquid medium and such enriched biomass use as a potential source of microelements in animal and/or human nutrition. It was found that the addition of different zinc forms, i.e. zinc nitrate, zinc sulphate and zinc chloride in fixed concentrations of 0, 25, 50 and 100 mg.100 ml^{-1} did not affect the amount of dry yeast biomass yielded, i.e. 1.0 – 1.2 g of yeast cells from 100 ml of cultivation medium, while higher presence of zinc solutions caused significantly lower yield of yeast biomass. The highest amount of zinc in yeast cells was achieved when added in the form of zinc nitrate in concentration of 200 mg.100 ml^{-1} YPD medium. The increment of intracellular zinc was up to 18.5 $mg.g^{-1}$ of yeast biomass.

Keywords: Zinc, *Saccharomyces cerevisiae*, Enriched biomass

INTRODUCTION

Saccharomyces cerevisiae is well-studied organism, which can serve as model system for the metals accumulation in fairly high concentrations; thus it is widely used in many branches of industry, i.e. biosorption, as a cost-effective biotechnology **(Wang and Chen, 2006; Chen and Wang, 2007)**, or in other part of industry (feed and food) because it is a high quality protein source rich in B-vitamins, amino acids and other various elements, i.e. Ca, Co, Fe, K and Na **(Swanson and Fahey, 2004; Fernandes et al., 1998)**. A very large number of proteins require metals for catalytic activity and/or for maintaining protein structure **(Waldron et al., 2009)**. More than 300 zinc enzymes are known, in which zinc play a regulatory role or is required for structure or catalytic activity. The uptake and accumulation of zinc by yeast is biphasic and consists of a metabolism-independent and a metabolism-dependent stage **(Bhagavan, 2002; Sekler et al., 2007; De Nicola et al., 2009)**.

This study aims to investigate the influence of Zn^{2+} on the yield of *Saccharomyces cerevisiae* biomass and presents the ability of zinc absorption into the cells.

MATERIAL AND METHODS

Biological material

Experiments were carried out with the yeast *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strain 612, which was obtained from the distillery Slovenské liehovary a likérky, a.s. Leopoldov, Slovakia. The yeast was maintained on Malt Extract agar for microbiology (Merck, Germany) and was grown on YPD (Yeast Peptone Dextrose) medium containing 10 $g.l^{-1}$ yeast extract (Imuna Pharm, Slovakia), 20 $g.l^{-1}$ peptone (Imuna Pharm, Slovakia) and 35 $g.l^{-1}$ glucose (Lachema, Czech Republic) with natural pH 6.6.

The condition of cultivation

Based on literature sources **(De Nicola et al., 2009; De Nicola and Walker, 2009; Chen and Wang, 2007; Stehlik-Tomas et al., 2004)** the stock solutions of zinc nitrate hexahydrate (pH 5.4), zinc sulphate heptahydrate (pH 4.6) and zinc chloride (pH 2.1) (Lachema, Czech Republic) were prepared in concentration of 10 % (w/w) in deionised water. Zn^{2+} was added to the required final concentrations of 25, 50, 100, 200 and 300 $mg.100\ ml^{-1}$,

respectively. Yeast cells were grown under condition of orbital shaker (MEZ, Czechoslovakia; 280 rpm) at 30 ˚C in dark for 48 h and initial cell densities of 0.5 x 10^6 cells.ml^{-1}. Yeast cell abundance was determined by a hemocytometer (Burker counting chamber, Meopta, Prague, Czech Republic). Biomass was harvested by centrifugation (K 70 D, Engelsdorf/Leipzig, Germany; 1500 x g, 30 min), rinsed twice with distilled water and lyophilized (LYOVAC GT 2, AMSCO/FINN-AQUA, Germany).

Sample preparation and AAS determination

The lyophilized yeast biomass was mineralized by concentrated nitric acid and distilled water (1:1) for 20 min at 160 °C in microwave oven (MarsXpress, CEM Corporation, USA). The obtained matter was filtrated via Filter Discs, Grade: 390, 84 $g.m^{-2}$, (Munktell and Filtrak Gmbh, Germany). The content of Zn^{2+} in yeast cell biomass was analysed by atomic absorption spectrophotometry method (AAS; Varian FS240, Varian company, Austria) using an air-acetylene flame, OD = 213.9 nm. Zinc lamp current: 8 mA.

Statistical evaluation

Results were expressed as the average values of six runs. Data were analyzed with an one-way analysis of variance (ANOVA) using **Statistica (2005)** and mean separation was performed with the Fisher's LSD test at P = 0.05. Data are presented as means ± SD.

RESULTS AND DISCUSSION

In this study the influence of zinc nitrate, zinc sulphate and zinc chloride on the yield of yeast biomass was determined and the differences among biosorptions of zinc forms by yeast cells of *S. cerevisiae* strain 612 were studied (Tab 1 – 3). The optimal concentration of zinc is specific for each yeast strain. Generally, for *S. cerevisiae*, 0.25–0.50 $\mu g.ml^{-1}$ appears to be optimal for cell growth **(De Nicola et al., 2009)**.

On the basis of results it was shown that concentrations of 0, 25, 50, 100 mg of all three zinc salts did not affect the yield of yeast biomass. It was obtained 0.99 – 1.19 g of dry yeast biomass from 100 ml YPD (Tab 1 – 3).

Table 1 Influence of zinc nitrate on the yield of yeast biomass and on the increment of zinc content in dry yeast biomass

Concentration of zinc nitrate (mg.100 ml^{-1} YPD)	Average yield of dry yeast biomass (g.100 ml^{-1} YPD)	Increment of Zn^{2+} content in dry yeast biomass (mg.kg^{-1})	Decrease of zinc content in medium after cultivation (%)
0	$1.18_a \pm 0.47$	$320.0_a \pm 16$	0
25	$1.13_a \pm 0.23$	$2400.0_b \pm 120$	10.8
50	$1.16_a \pm 0.25$	$4440.0_c \pm 222$	10.3
100	$1.04_a \pm 0.25$	$8100.0_d \pm 405$	8.4
200	$0.14_c \pm 0.48$	$18500.0_e \pm 925$	1.3
300	$0.01_b \pm 0.01$	-	-

Legend: $P < 0.05$, values are means ± SD, n = 6. YPD – Yeast Peptone Dextrose medium

Table 2 Influence of zinc sulphate on the yield of yeast biomass and on the increment of zinc content in dry yeast biomass

Concentration of zinc sulphate (mg.100 ml^{-1} YPD)	Average yield of dry yeast biomass (g.100 ml^{-1} YPD)	Increment of Zn^{2+} content in dry yeast biomass (mg.kg^{-1})	Decrease of zinc content in medium after cultivation (%)
0	$1.18_a \pm 0.47$	$320.0_a \pm 16$	0
25	$1.17_a \pm 0.16$	$3820.0_b \pm 191$	17.8
50	$1.13_a \pm 0.19$	$5800.0_c \pm 290$	13.1
100	$0.99_a \pm 0.14$	$9440.0_d \pm 472$	9.3
200	$0.006_b \pm 0.01$	-	-
300	$0.01_b \pm 0.01$	-	-

Legend: $P < 0.05$, values are means ± SD, n = 6. YPD – Yeast Peptone Dextrose medium

Table 3 Influence of zinc chloride on the yield of yeast biomass and on the increment of zinc content in dry yeast biomass

Concentration of zinc chloride (mg.100 ml^{-1} YPD)	Average yield of dry yeast biomass (g.100 ml^{-1} YPD)	Increment of Zn^{2+} content in dry yeast biomass (mg.kg^{-1})	Decrease of zinc content in medium after cultivation (%)
0	$1.18_a \pm 0.47$	$320.0_a \pm 16$	0
25	$1.19_a \pm 0.69$	$3280.0_b \pm 164$	15.6
50	$1.17_a \pm 0.05$	$5540.0_c \pm 277$	12.9
100	$0.99_a \pm 0.15$	$10380.0_d \pm 519$	10.3
200	$0.03_b \pm 0.03$	-	-
300	$0.04_b \pm 0.01$	-	-

Legend: $P < 0.05$, values are means ± SD, n = 6. YPD – Yeast Peptone Dextrose medium

The higher concentrations (200 and 300 mg) caused death of yeast cells (P = 0.000). Only when zinc nitrate was applied in the concentration of 200 mg.100 ml^{-1}, small amount of yeast biomass was obtained. Higher concentrations of zinc chloride (200 and 300 mg.100 ml^{-1}) added into the cultivation medium affected decreasing of yield of biomass (P = 0.000). Optimum pH for zinc biosorption by the yeast is 5.8 **(Chen and Wang, 2007)**. pH of YPD medium used in these experiments was not adjusted. It was changed by addition of different zinc solutions and ranged between 5.7–6.6. Cellular Zn^{2+} occurrence became significantly increased (P = 0.000) according to the level of zinc addition into the cultivation medium. The highest increment of Zn^{2+} concentration in yeast cells was determined after cultivation in YPD medium with addition of 200 mg of zinc nitrate in 100 ml YPD which represented 18.5 $mg.g^{-1}$.

Eide (2003) supposes, that mechanism of transport proteins in the plasma membrane maintains intracellular zinc at extremely low levels. **Stehlik-Tomas et al. (2004)** and **Poreda et al. (2009)** reported the highest amount of zinc ions in dry matter of yeast biomass 0.6 – 0.7 $mg.g^{-1}$.

Zinc deficiency in organism is a cause of diseases and determinates its progress **(Abbas, 2006; Maret and Sandstead, 2006)**. Among these nutritional importance, i.e. bioavailability of divalent minerals such as zinc **(Sereih et al., 2011)** this study also confirmed the potentiality of yeast as an alternative for bioremediation of heavy metal from polluted waters **(Bishnoi and Garima, 2005)**.

CONCLUSION

This study confirmed the suitability of yeast as a supplement in nutrition for their nutraceutical or health-promoting attributes. It was confirmed, that zinc was absorbed from the cultivation medium by yeast cells regularly in accordance with the level of zinc addition into the medium. The highest amount of zinc in yeast cells (18.5 $mg.g^{-1}$) was achieved when added in the form of zinc nitrate in concentration of 200 mg.100 mL^{-1} of YPD medium.

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