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BIOMASS OF *CANDIDA UTILIS* ATCC 9950 YEAST AS A SOURCE OF MAGNESIUM BIOPLEXES

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The study was aimed at investigating the possibility of obtaining a natural bioplex of magnesium from the biomass of fodder yeast *C.utilis* ATCC 9950 enriched in that element. Yeast cultures were run under dynamic conditions (with aeration) for 48 h on a control medium (YPD) and the experimental one enriched in magnesium ions at a concentration of 1.25 g/L. During the logarithmic phase of growth, magnesium was observed to bind the mannoprotein layer of the cell wall of yeast (68% of the total magnesium in biomass), whereas in the stationary phase it was subject to intracellular bioaccumulation. Consequently, 61% of the cellular magnesium pool were accumulated in protoplasts and 39% in cell walls. The bioplex separated from the 48-h experimental culture contained nearly 3-fold more magnesium (8.51 mg Mg/g d.s.) than that isolated from the control culture (2.92 mg Mg/g d.s.).

INTRODUCTION

Recent years have brought about substantial changes in nutrition patterns, as a result of which the interrelations between health and food have acquired a special significance. Nowadays, it is claimed that the basis of maintaining good health and preventing civilization diseases is a well balanced diet. Very often highly-processed food is depleted of multiple valuable minerals, including magnesium, which in turn leads to magnesium deficiency in the organism. It is estimated that magnesium deficiency affects 50%–80% of the Polish population [Brzozowska, 1998]. The pharmaceutical market offers ready preparations containing magnesium in the form of non-organic salts. Yet, their application has failed to bring the expected results mainly due to a low availability [Świątkiewicz & Korelski, 1998]. Ample studies have proved that better availability of micro- and macroelements occurs in the case of organic links of those elements with *e.g.* proteins, compared to the non-organic ones [Close, 1998; Spears, 1996; Vandergrift, 1991].

Yeast capacity for binding cations present in the culture medium affords the possibility of obtaining bioplexes of those elements whose deficiency in human and animal diets requires pharmacological supplementation [Błażejak & Duszkiewicz-Reinhard, 2004; Gardner, 2003; Graschopf *et al.*, 2001; Lipke & Ovalle, 1998; MacDiarmid & Gardner, 1998]. The elimination of magnesium deficiency in humans through the administration of bioplexes, which easily penetrate the hydrophobic layer of cytoplasmatic membrane, may be an alternative source of dietary magnesium to the commonly used, yet little available pharmacological preparations.

Results of investigations carried out so far at the Department of Food Biotechnology and Microbiology, Warsaw Agricultural University [Błażejak *et al.* 2002, 2003a, b], on yeast strains of the *Saccharomyces* and *Candida* genera have indicated the possibility of magnesium binding by cells of those fungi from experimental medium in the amounts considerably exceeding physiological demands for that element. A part of magnesium ions (40–60% of their total content in cells) could have been removed from the cells through washing the yeast biomass with deionised water. That readily-removable magnesium was present in the intercellular spaces and, probably, bound with extracellular exopolysaccharides. The other part was constituted by magnesium permanently bound with yeasts, especially with mannoproteins of the cell wall that contain ligands capable of binding bivalent cations of magnesium from the culture medium.

In this study, it was assumed that magnesium adsorbed in the mannoprotein layer of the cell wall during the logarithmic growth phase of yeasts on the experimental medium enriched with that element was subject to intracellular bioaccumulation, which afforded the possibility of generating its bioplexes.

The aim of the study was to obtain a natural magnesium bioplex from cell biomass of fodder yeast *Candida utilis* ATCC 9950 cultured under dynamic conditions on experimental media enriched with chloride salt of that element.

The first stage of investigations addressed: the evaluation of magnesium and protein distribution in cells of the yeast strain examined; the determination of the effect of Mg ions dose applied in control medium on magnesium content of biomass and cell wall of yeast; and determination of the

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cellular pool of magnesium permanently bound by yeasts in protoplasts. Taking into account the potential impact of an increased magnesium concentration in the culture medium on the biosynthesis of cellular proteins, their content was determined in cell walls and protoplasts and their quality was evaluated after electrophoretic separation on polyacrylamide gels with SDS. In addition, the effect of the applied dose of magnesium ions on cell morphology of the strain under study was verified.

The second stage of investigations involved the separation of bioplexes from *Candida utilis* cell biomass obtained from the control (without Mg ions) and experimental cultures (enriched with Mg ions) and their characteristics in terms of magnesium content.

MATERIAL AND METHODS

Biological material. The experimental material included a strain of fodder yeast *Candida utilis ATCC 9950* originated from a pure culture collection of the Department of Food Biotechnology and Microbiology, Warsaw University of Agriculture, Warsaw, Poland.

Microbiological media. The following media were used in the study: (i) control medium – liquid YPD medium [Suizu *et al.*, 1994]; and (ii) experimental medium – liquid YPD medium enriched with Mg ions; magnesium was added as $MgCl_2 \cdot 6H_2O$ salt at a dose providing its content in medium at a level of 1.25 g/L.

Yeast culture on control and experimental media. In each series of the experiment, control and experimental media were inoculated with 10% (v/v) of 24-h *inoculum*. The cultures were run for 48 h on a reciprocating shaker (SM-30 Control E. Bühler, Niemcy) with vibration frequency of 200 cycles per minute at a temperature of +28°C.

Measurement of optical density (OD). In each experimental series, the *inoculum* culture was carried out until OD of *ca.* 2.00 has been reached, due to which the cell number of yeast introduced with 10% (v/v) *inoculum* into the control and experimental media was alike.

The optical density of the *inoculum* was measured with a spectrophotometer (Spectronic 20 Genesys, USA) at a wave length of 600 nm.

Cell biomass preparation for the determination of protein and magnesium contents of yeasts and yeast cell walls. Samples to be determined for the contents of protein and magnesium were collected from control and experimental media in the following time intervals: T0 – immediately after the introduction of *inoculum*, as well as T24 and T48. The samples were centrifuged for 10 min at 3500 rpm (Centrifuge type MPW – 365, Poland), then supernatant was decanted from above the precipitate. In order to remove residues of culture medium and magnesium ions not bound with yeast cells, the biomass obtained was rinsed with deionised water and once again centrifuged. Next, the biomass was dried at a temperature of +80°C (Zelmed SML 32/250, Poland) to a constant weight.

So as to prepare the samples for the determination of protein and magnesium contents of the cell wall of *Candida utilis* yeasts, the cells well mechanically disintegrated at a temperature of liquid nitrogen. Centrifuged cell biomass of yeasts from particular cultures was transferred into a ceramic mortar and ground in liquid nitrogen (-196°C). The ground biomass was transferred to a thimble, rinsing the mortar several times with deionised water, and again centrifuged. It was assumed that the precipitate contained fragments of cell walls. The precipitates were dried at a temperature of +80°C to a constant weight.

Isolation of magnesium complex from cell biomass of yeasts. Magnesium bioplex was obtained from cell biomass of fodder yeasts *Candida utilis* ATCC 9950 cultured for 24 h (T24) and 48 h (T48) under dynamic conditions on control YPD medium and the experimental one enriched with Mg^{2+} ions at a dose of 1.25 g/L.

Cell biomass of individual cultures was separated from the medium by centrifugation (3500 rpm for 10 min) in a centrifuge with cooling at a temperature of +4°C (Eppendorf Centrifuge 5804 R, USA). Supernatant was removed and precipitate was rinsed with sterile deionised water and centrifuged once more (at the above-mentioned parameters). The centrifuged biomass was suspended in deionised water by preparing a suspension with a density of 3°Blg, then proteins were extracted under conditions of base hydrolysis. This method consisted in alkalizing the suspension of yeast cells to pH of *ca.* 11.5 with NaOH and heating it to a temperature of *ca.* 60°C for 30 min on a magnetic stirrer (ES 21H Electromagnetic stirrer, Poland) [Parajo *et al.*, 1995a, b].

After the alkaline extraction, the suspension was cooled to a room temperature and next centrifuged at a temperature of +4°C. The supernatant solution obtained contained hydrolysed components of yeast cell biomass.

The next stage of investigations involved the isolation of a magnesium bioplex from a solution. To this end, pH of the supernatant was decreased with 6 mol/L HCl to a value of *ca.* 4.5 (*i.e.* isoelectric point of yeast proteins), after which cell proteins were observed to precipitate. At the isoelectric point, the solution was cooled to a temperature of 0–4°C, and the extracted components of cell biomass present therein were additionally precipitated with ethanol. The alcohol was added to the solution at a ratio of 2:1 (v/v), as a result of which the bioplex fell out in the form of a precipitate [Stasińska, 1999]. The solution with the precipitated bioplex was centrifuged at 3500 rpm for 10 min, the supernatant was decanted and the precipitate dried at a temperature of +80°C to a constant weight.

The dried and weighed precipitates of bioplexes obtained from the control and experimental media after 24- and 48-h culture of *Candida utilis* yeasts were determined for the content of magnesium with Atomic Absorption Spectrometry (AAS).

Determination of magnesium content of biomass and cell wall of yeasts. Dried and weighed samples of yeast cell biomass or cell wall fragments were mineralized in a mixture of 5 mL of 65% HNO_3 and 2 mL of 70% $HClO_4$ in a

digestion unit (Büchi Digestion Unit K-435, Germany), with a heating panel temperature of +600°C. After the mineralization, the content of thimbles was quantitatively transferred to measuring flasks to a volume of 25 mL, rinsing the thimbles with 10% HCl. The content of magnesium was determined with Atomic Absorption Spectrometry (AAS) on an absorption spectrometer (Shimadzu AA-660, Japan) at a wave length of 285.2 nm [Bryłka *et al.*, 1995].

Magnesium content of biomass and cell wall samples (after considering their percentage in *C. utilis* cells) was expressed as mg Mg/g d.s. of yeasts.

Determination of total nitrogen in cell biomass and cell wall of yeasts. Determination of the content of total nitrogen in the samples of yeast biomass or fragments of cell walls after cell disintegration was carried out following the Kjeldahl's method according to Polish Standard [PN-75/A--04018] after prior mineralization of the samples (Büchi Digestion Unit K-435, Germany) and distillation of ammonia [Klepacka *et al.*, 2000]. Nitrogen content of biomass and cell wall samples (after considering their percentage in *C. utilis* cells) was converted into total protein and expressed as g protein/100 g d.s. of yeasts.

Isolation of proteins from cell biomass of yeasts. Yeast proteins were isolated from control and experimental cultures with a commercial apparatus Y-PER® (Yeast Protein Extraction Reagent No. 78991) by the Pierce company. The procedure of cellular protein separation from yeasts was carried out in the following steps: (i) centrifugation of biomass from 5 mL of yeast culture at a temperature of +4°C, at 5000 rpm for 5 min; (ii) removal of the supernatant and addition of 2.5 mL of Y-PER® preparation to the biomass obtained; (iii) incubation of the samples at a room temperature for 20 min; and (iv) centrifugation at 11 000 rpm for 10 min; collection of the supernatant and its electrophoresis.

Electrophoretic separation of proteins on polyacrylamide gel with SDS. In order to characterize cellular protein fractions isolated from fodder yeasts *Candida utilis*, their electrophoretic separation was carried out on polyacrylamide gel with SDS [Maniatis *et al.*, 1989]. The electrophoresis was conducted on protein solutions isolated from 24- and 48-h cultures of yeasts on control and experimental medium as well as on standard weights (Precision Plus Protein Standards, Bio-Rad) with the known molecular weights of: 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa.

In the separation, use was made of 4% thickening gel and 12% separating gel. The electrophoresis was carried out on an Electrophoresis System Model P100DS apparatus with cooling by Owner's Manual (USA). On the thickening gel the process was carried out at a current intensity of 20 mA (for *ca.* 30 min), whereas on the separating gel – at a current intensity of 40 mA (for *ca.* 1.5 ha), and voltage not exceeding 250 V at the end of the separation process. On electrophoresis completion, striae were fixed on gels in 20% (w/v) solution of TCA (tricholoracetic acid) and stained with 0.2% (w/v) solution of Coomassie Brilliant Blue R-250.

Protein fractions separated electrophoretically on polyacrylamide gel were analysed in respect of their molecular weights with Quantity One software (France).

Determination of the effect of magnesium on yeast morphology. The effect of the magnesium dose administered to the culture medium on the cell morphology of fodder yeasts *Candida utilis* ATCC 9950 was investigated.

Microscope preparations were prepared from 24- and 48-h cultures on control and experimental media, that were next photographed with a digital camera (Sony, DSC-85S, Japan) mounted on a microscope (Zeiss Axiostar Plus, HBO-50/AC). Pictures were taken at 1600x magnification. Measurements of the size area as well as the length and width of yeast cells and vacuoles were carried out by means of LSM 5 Image Browser software (Germany).

Statistical analysis. Mean results of determinations of 7 experimental series (each in 3 replications) and measurements of the size area of 100 yeast cells were subjected to a statistical analysis with Statgraphics Plus ver. 4.1 software. An analysis of variance and the Tukey's test at a significance level of $\alpha = 0.05$ were carried out as well.

RESULTS AND DISCUSSION

The culture of fodder yeast *Candida utilis* was run under dynamic conditions at the control YPD medium and the experimental one supplemented with 1.25 g Mg/L in the form of chloride salt. The selection of both the source and dose of magnesium resulted from previous investigations carried out at the Department of Food Biotechnology and Microbiology, Warsaw University of Agriculture [Błażejak *et al.*, 2002, 2003a, b].

Magnesium and protein contents of the yeast cell biomass were determined in the samples from control and experimental media in time intervals of T0, T24 and T48. The contents of magnesium and protein were assayed in both the whole cells and cell walls of *Candida utilis* yeasts after their mechanical disintegration at a temperature of liquid nitrogen. A bioplex of magnesium was obtained from 24- and 48-h cultures run under dynamic conditions on control and experimental media and determined for magnesium content.

So as to determine the effect of Mg ions in the experimental medium on protein synthesis and to elucidate the mechanism of magnesium bioaccumulation in yeasts to a more explicit extent, proteins isolated from cell biomass of fodder yeasts *Candida utilis* were subjected to electrophoretic separation.

The size of the cells and the coefficient of their shape were determined as well, which enabled recognizing to what extent the magnesium dose applied in the culture medium altered the morphology of *Candida utilis* compared to the cells cultured on standard YPD medium.

The first stage of investigations was aimed at determining the capability of *C. utilis* cells to bind magnesium during 48-h culture on control and experimental media.

In time T0, *i.e.* immediately after *inoculum* introduction to the media, the yeasts were observed to bind similar amounts of magnesium (Tables 1 and 2). The percentage

TABLE 1. Changes in magnesium content (mg Mg/g d.s.) of *Candida utilis* ATCC 9950 yeasts from control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Time of culture (h)	YPD (control)	YPD+1.25 g Mg^{2+}/L
0	$1.82 \pm 0.11^{b*}$	1.85 ± 0.12^{b}
24	1.92 ± 0.12^{c}	5.17 ± 0.22^{d}
48	1.42 ± 0.10^{a}	6.14 ± 0.25^{e}

* – the same letter index means a lack of significant difference

TABLE 2. Changes in magnesium content (mg Mg/g d.s.) of the cell walls of *Candida utilis* ATCC 9950 yeasts from control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Time of culture (h)	YPD (control)	YPD+1.25 g Mg^{2+}/L
0	$1.37 \pm 0.10^{c*}$	1.35 ± 0.11^{c}
24	0.64 ± 0.05^{b}	3.54 ± 0.19^{e}
48	0.41 ± 0.04^{a}	2.42 ± 0.14^{d}

* – the same letter index means a lack of significant difference

distribution of the entire magnesium pool between cell walls and protoplasts indicated that in the cells from the control media 76% of total magnesium were accumulated in the cell walls and 24% in the protoplasts (Table 3). A similar distribution of magnesium (73% in the cell walls and 27% in the protoplasts) was reported for the cells from the experimental medium (Table 3).

Time T0 appeared to be too short to allow the yeast cells to bind greater amounts of magnesium from the experimental medium even at its supplementation with an increased concentration of magnesium.

After 24-h culture (T24), a significant increase was observed in magnesium content of cells from both the control and experimental medium (Tables 1 and 2). The yeasts from the medium enriched with magnesium ions contained 3.54 mg Mg/g d.s. in the cell walls and 1.63 mg Mg/g d.s. in the protoplasts, on average (Table 3). In the same time interval, cells of the control medium bound on average 0.64 mg Mg/g d.s. in the cell walls and 1.29 mg Mg/g d.s. in the protoplasts. In the yeast from the control culture, the percentage distribution of the total cellular magnesium pool in time T24 was as follows: 33% in the cell walls and 67% in the protoplasts (Table 3). In the case of the yeasts from the experimental cultures, 68% of magnesium were located in the cell walls and 32% in the protoplasts.

The highest average content of magnesium – 6.14 mg Mg/g d.s. – was reported for the cells of the experimental media after 48-h culture (Table 1), including 2.42 mg of magnesium accumulated in the cell walls and 3.72 mg in the protoplasts (Table 3). In the same time interval, the yeasts from the control medium were capable of binding as little as 1.42 mg Mg/g d.s. (including 0.41 mg of magnesium in the cell walls and 1.01 mg in the protoplasts). In the yeast cells from the standard YPD medium, 70% of magnesium were accumulated in the protoplasts and 30% in the cell walls (Table 3). In a comparable time span, the yeast cells of the experimental medium accumulated 61% of the total magnesium in the protoplasts and 39% in the cell wall. The increased percentage of magnesium in the protoplasts after 48 h of culture accompanied by a decline in magnesium percentage in the cell walls may point to intracellular bioaccumulation of that element during the stationary growth phase of the fodder yeast strain examined [Brady & Duncan, 1994; Park *et al.*, 2003; Tuszyński, 2000].

The results obtained indicate that during the growth on culture media the fodder yeasts *Candida utilis* activated the mechanisms of magnesium transport into the cells' interior after magnesium supplies in the medium have been depleted. Reduced magnesium content of the control YPD medium caused that the intracellular bioaccumulation of Mg ions was observed as early as after 24 h of the culture.

In the case of the magnesium-supplemented experimental media, a similar phenomenon occurred after 48-h culture. The increased concentration of magnesium in the medium naturally extended the time of chemisorption of that ion in the mannoprotein layer of the cell wall. Only then was the intracellular accumulation of Mg ions observed, which enabled the formation of magnesium bioplexes.

The next stage of investigations was aimed at determining the effect of magnesium dose applied in the experimental medium on the total synthesis of proteins by the strain *C. utilis* during 48-h culture.

A considerable increase in protein content was observed in time T24 in yeast cells from the experimental cultures (Tables 4-6). The average protein content of yeasts from the experimental medium reached 61.2 g/100 g d.s. (including 11.5 g in the cell walls and 49.7 g in the protoplasts). In the same time interval, the cells of the control medium contained 53.8 g of protein/100g d.s. on average (Table 4), including 9.66 g of protein in the cell walls and 48.12 g in the protoplasts (Table 6).

After two days of culture, protein contents of the yeasts from both control and experimental cultures were subject to a slight but significant decrease, which was likely to result from intensified processes of cell autolysis in the phase of stationary growth (Tables 4 and 5).

The percentage distribution of the total cellular protein pool between protoplasts and cell walls in time T48 differed

TABLE 3. Changes in magnesium content (mg Mg/g d.s.) of the protoplasts of *Candida utilis* ATCC 9950 yeasts from control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Medium Time of culture (h)	Cell biomass	Cell wall	Protoplast	Percentage distribution of magnesium (%)	
				Cell wall	Protoplast
YPD (control)					
0	1.82	1.37	0.43	76	24
24	1.92	0.64	1.29	33	67
48	1.42	0.41	1.01	30	70
YPD+1.25 g Mg^{2+}/L					
0	1.85	1.35	0.50	73	27
24	5.17	3.54	1.63	68	32
48	6.14	2.42	3.72	39	61

TABLE 4. Changes in total protein content (g/100 g d.s.) of *Candida utilis* ATCC 9950 yeasts from control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Time of culture (h)	YPD (control)	YPD+1.25 g Mg^{2+}/L
0	$53.2 \pm 1.2^{b*}$	53.2 ± 1.2^{b}
24	53.8 ± 1.3^{b}	61.2 ± 0.9^{d}
48	52.6 ± 1.1^{a}	60.1 ± 1.0^{c}

* – the same letter index means a lack of significant difference

TABLE 5. Changes in total protein content (g/100 g d.s.) of the cell walls of *Candida utilis* ATCC 9950 yeasts from control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Time of culture (h)	YPD (control)	YPD+1.25 g Mg^{2+}/L
0	$8.8 \pm 0.4^{b*}$	8.8 ± 0.4^{b}
24	8.9 ± 0.4^{b}	11.5 ± 0.5^{d}
48	7.5 ± 0.4^{a}	10.0 ± 0.5^{c}

* – the same letter index means a lack of significant difference

from that reported in the cells in time T24 (Table 6). *Candida utilis* cultured for 48 h on the medium enriched with Mg ions contained on average 83% of total protein of cell biomass in the protoplasts and 17% in the cell walls. In the yeasts from the control media, the percentage distribution of protein pool was as follows: 86% in the protoplasts and 14% in the cell walls. The higher percentage of proteins in the cell walls of the yeasts from the experimental medium compared to those from the control culture might have resulted from the protective function of magnesium for cellular structures [Birch & Walker, 2000; Hartwig, 2001].

The results obtained enable assuming that the increased protein content accompanied by the increased concentration of magnesium in the cells of yeasts cultured on experimental media could have been linked with the expression of genes responsible for the synthesis of protein carriers of Mg ions into the cells' interior based on facilitated diffusion or active transport [Gadd, 2004; Gardner, 2003; Graschopf *et al.*, 2001].

Bioplexes are organic links of micro- or macroelements with organic polymers (mainly proteins) whose addition to food may eliminate deficiencies of scarce minerals [Matyka & Korol, 1997]. At this stage of the experiment, under the conditions of base hydrolysis, magnesium bioplexes were isolated from the biomass of fodder yeasts obtained from the control and experimental cultures.

The results obtained (Table 7) indicated that the addition of Mg ions to the culture medium had a significant effect on the content of magnesium in the bioplex obtained. In time T24, the bioplex of the control medium contained

TABLE 7. Magnesium content (mg Mg/g d.s.) of the bioplex obtained from cell biomass of *Candida utilis* ATCC 9950 yeasts cultured under dynamic conditions on control medium and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Time of culture (h)	YPD (control)	YPD+1.25 g Mg^{2+}/L
24	$2.30 \pm 0.14^{a*}$	6.95 ± 0.20^{c}
48	2.92 ± 0.12^{b}	8.51 ± 0.34^{d}

* – the same letter index means a lack of significant difference

on average 6.95 mg Mg/g d.s., whereas that of the control YPD medium – as little as 2.30 mg Mg/g d.s. In time span T48, the values accounted for 8.51 mg Mg/g d.s. and 2.92 mg Mg/g d.s., respectively.

The results obtained demonstrated the possibility of natural enrichment of *Candida utilis* yeast cells in magnesium under dynamic conditions. In addition, a significant increase in the content of that element was obtained in the isolated bioplex due to the permanent binding of Mg ions with the components of the yeast cell biomass.

The results of the experiment prompted us to carry out the electrophoresis of cellular proteins isolated from the

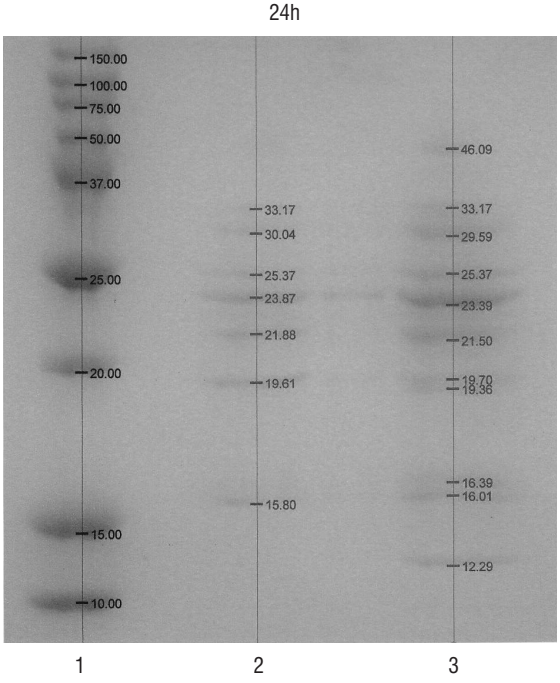

FIGURE 1. Electrophoregram (12% polyacrylamide gel with SDS) of proteins of *C. utilis* yeasts from 24-h control and experimental cultures. Explanations: 1 – proteins with the known molecular weight; 2 – proteins of yeasts from 24-h control culture; 3 – proteins of yeasts of 24-h experimental culture

TABLE 6. Changes in total protein content (g/100 g d.s.) of the protoplast and cell wall of *Candida utilis* ATCC 9950 yeasts from control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Medium Time of culture (h)	Cell biomass	Cell wall	Protoplast	Percentage distribution of magnesium (%)	
				Cell wall	Protoplast
YPD (control)					
0	53.2	8.8	44.4	17	83
24	53.8	8.9	44.9	17	83
48	52.6	7.5	45.1	14	86
YPD+1.25 g Mg^{2+}/L					
0	53.2	8.8	44.4	17	83
24	61.2	11.5	49.7	19	81
48	60.1	10.0	50.1	17	83

biomass of *C. utilis* yeasts aimed at their qualitative identification. The electrophoretic separation on 12% polyacrylamide gel with SDS was conducted on protein solutions isolated from 24- and 48-h yeast cultures (Figures 1 and 2).

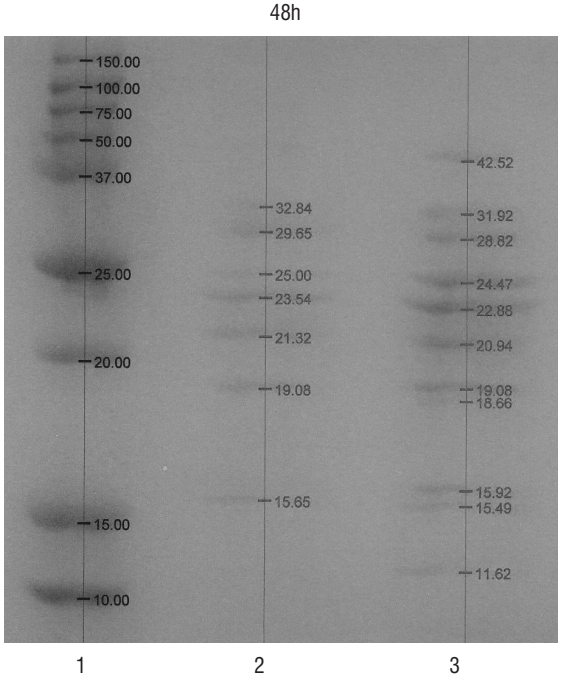

FIGURE 2. Electrophoregram (12% polyacrylamide gel with SDS) of proteins of *C. utilis* yeasts from 48-h control and experimental cultures Explanations: 1 – proteins with the known molecular weight; 2 – proteins of yeasts from 48-h control culture; 3 – proteins of yeasts of 48-h experimental culture

In the molecular weight range of 37–20 KDa, the number of protein fractions isolated from the cell biomass of the yeasts cultured under control and experimental conditions was the same, and their molecular weights were very similar. Differences occurred in the proteins with molecular weights ranging from 20 KDa to 10 KDa. As postulated by Gwóźdź & Kopyra [2003] and Truchliński & Pasternak [2002], proteins with molecular weight below 20 KDa, rich in cystein (of metalothionein or phytochalatin type), may participate in the intracellular transport of cations. The yeast cells from T24 (Figure 1) and T48 (Figure 2) cultured on the magnesium-supplemented experimental medium were observed to contain a higher number of protein fractions of that range, compared to the yeast cells cultured under control conditions. In addition, on electrophoregrams from the experimental cultures there appeared protein fractions with the molecular weight over 40 KDa, that were not detected in the yeasts of the control cultures. In time intervals T24 and T48, the striae originating from the proteins isolated from the yeasts of experimental cultures had a more intensive colour than those of the control cultures, which may indicate a higher concentration of those fractions in the cells of magnesium-enriched yeasts (Figures 1 and 2).

A comparative analysis of the electrophoretic separations of proteins isolated from fodder yeasts C. utilis cultured on experimental media enriched with 1.25 g/L of magnesium and from those cultured on control YPD medium points to the intensification of protein biosynthesis during the logarithmic phase of growth (T24). In the stationary phase (T48), the number of isolated protein fractions remained unchanged, and some of them (with molecular weights below 20 KDa) might have participated in the intracellular accumulation of magnesium in the form of bioplexes.

The final stage of the experiment was aimed at determining to what extent the magnesium dose applied in the experimental medium affected the morphology of *C. utilis* cells. The measurements performed (Table 8) indicated that in time intervals T24 and T48, the cells from the experimental cultures were significantly smaller than those from the control cultures, which could have been due to a shorter generation time on media enriched with magnesium or to the effect of increased osmotic pressure. The other morphological parameters of the cells from the control and experimental cultures, including the size of vacuoles and shape coefficient, were not significantly different in both T24 and T48.

It seems that the magnesium addition applied to the culture medium did not disturb the natural metabolic activity of the fodder yeast strain examined. As reported by Park *et al.* [2003], the intracellular biosorption of cations proceeds mainly in yeast vacuoles. The concentration of Mg ions in those organelle is 35 times higher than in cytoplasma [Mochaba *et al.*, 1997]. A lack of significant changes in the size of vacuoles of the yeasts from experimental cultures compared to those of the control cultures confirms the possibility of magnesium occurrence in the form of bioplexes (Table 8).

Contrary to mineral salts, in the water medium magnesium bioplexes do not produce free cations [Matyka & Korol, 1997]. Probably for this reason, the size of vacuoles was not subject to significant changes even at nearly 3-fold higher content of magnesium in the protoplasts of yeast from 48-h experimental culture, compared to the control one.

CONCLUSIONS

1. It is possible to obtain a natural bioplex of magnesium

TABLE 8. Changes in cell morphology of *Candida utilis* ATCC 9950 yeasts after 24- and 48-h control culture and the experimental one enriched with $MgCl_2 \cdot 6H_2O$.

Medium	Time of culture (h)	Cell size area Csa (μm^2)	Vacuole size area Vsa (μm^2)	Cell length l (μm)	Cell width D (μm)	Vsa/Csa	Shape coefficient l/d
YPD (control)	24	24.88±1.50a*	8.67±0.52a*	7.59±0.45a*	3.94±0.21a*	0.35	1.93
YPD+1.25gMg^{2+}/L		23.04±1.37b	8.71±0.51a	7.36±0.42b	3.84±0.20a	0.38	1.92
YPD (control)	48	31.79±1.58a*	5.89±0.35a*	8.27±0.41a*	4.98±0.29a*	0.19	1.66
YPD+1.25gMg^{2+}/L		28.61±1.43b	6.39±0.31a	7.87±0.39b	4.72±0.22b	0.22	1.66

* – the same letter index means a lack of significant difference

with an increased content of that element from cell biomass of fodder yeasts *Candida utilis* ATCC 9950.

2. The excess of magnesium in the culture medium exceeding the physiological demand of yeast cells leads to the chemisorption of that element in the cell wall of yeasts during the logarithmic growth phase and to its intracellular bioaccumulation in the stationary phase.

3. The magnesium dose applied in the experimental medium (1.25 g/L) was found to have a significant effect on enhanced synthesis of proteins in the cells of *C. utilis* yeasts, compared to the cells from the control YPD medium, and on the appearance of a higher number of protein fractions with molecular weights lower than 20 KDa that may be responsible for magnesium transport into the cells' interior and participate in the formation of bioplexes.

4. The magnesium dose applied in the experimental media was also observed to significantly affect decreasing sizes of the cells, compared to the yeasts from the control culture. Other morphological parameters, including the size of vacuoles and coefficient of cell shape, remained unchanged, which indicates the occurrence of magnesium in the form of protein complexes.

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BIOMASA DROŻDŻY *CANDIDA UTILIS* ATCC 9950 JAKO ŹRÓDŁO BIOPLEKSÓW MAGNEZU

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Badano możliwość otrzymania naturalnego biopleksu magnezu z biomasy drożdży paszowych *C.utilis* ATCC 9950 wzbogaconych w ten pierwiastek. Hodowle drożdży prowadzono metodą wgłębną przez 48 godzin na podłożu kontrolnym (YPD) i doświadczalnym wzbogaconym jonami magnezu w stężeniu 1,25 g/dm^3. Podczas logarytmicznej fazy wzrostu magnez wiązał się z mannoproteinową warstwą ściany komórkowej drożdży (68% całkowitej ilości magnezu zawartego w biomasie), natomiast w fazie stacjonarnej ulegał wewnątrzkomórkowej bioakumulacji. W rezultacie 61% komórkowej puli magnezu znajdowało się w protoplastach, a 39% w ścianach drożdży (tab. 3). Wyodrębniony biopleks z 48-godzinnej hodowli doświadczalnej zawierał prawie 3-krotnie więcej magnezu (8,51mg Mg/g s.s.) niż z hodowli kontrolnej (2,92 mg Mg/g s.s.) (tab. 7).