ISSN : 0974 - 7435 Volume 10 Issue 23

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(23), 2014 [14080-14087]

Preparation of high-biomass chromium-enriched *saccharomyces cerevisiae* cr-y yeast

Min Zhang[1,2], Jianhong Yang[1], Jie Zhang, Xi Li[2], Wenjun Ding[1*]

[1]Department of Biological and Environmental Engineering, Hefei University, (CHINA)

[2]School of Life Sciences, Graduate University of Chinese Academy of Sciences, (CHINA)

E-mail: dingwj@gucas.ac.cn

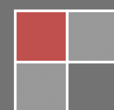
ABSTRACT

To obtain high chromium-collected Saccharomyces Cerevisiae, the optimal conditions for a strain of *Saccharomyces cerevisiae* N-Y which could tolerate high concentration of chromium were investigated. The optimal conditions for the N-Y by single factor and orthogonal array design methods was as follows: 30 g/L gucrose, 5 g /L $NaNO_4$, 20 g/L yeast extract, 28~40℃, pH4~6, rotation speed at 200 rpm or more than 200 rpm. Subsequently, the N-Y was cultured in the optimal media and conditions with 3200 μg/ml chromium acetate acid for different time and was transfered at least 30 passages. We got a strain of *Saccharomyces cerevisiae* named Cr-Y which could collect chromium as 500 μg per gram dried yeast. The chromium-riched could reduce the sugar of diabetes mellitus type I animal model produced by steptotozocin obviously. The chromium collected from the Cr-Y was in the yeast, while other reported resulted suggested that part of the chromium was out of the yeast. The results of the sugar-reducing effects of our Cr-Y had been reported in other journal. The content of chromium was detected by ICP-MS.

KEYWORDS

Chromium-rich yeast; Optimal medium; ICP-MS; High-biomass.

© Trade Science Inc.



INTRODUCTION

Chromium (Cr) especially organic chromium, as an essential element, is thought to play a crucial role in normal carbohydrate and lipid metabolism by potentiating the action of insulin, and, as early as 1957, it was already being referred to as “a glucose tolerance factor”[1]. In vitro and in vivo investigations have shown that chromium can improve insulin resistance[2-3]. Although toxic effects were reported in neither the systematic reviews[4-5] nor in Anderson et al.’s study[6], chromium picolinate was banned by the Food Standards Agency until December of 2004[7]. This meant that investigations into the effects of chromium compounds on type 2 diabetes had to involve compounds other than chromium picolinate such as organic chromium in *Saccharomyces cerevisiae*. Some studies, which investigated the effects of chromium-enriched yeast in non-diabetic patients, showed mixed results[8-11]. Machado *et al* reported that the *Saccharomyces cerevisiae* could be used to remove chromium, copper, and nickel from Electroplating effluent[12].

The glucose tolerance factor (GTF) is a dietary agent that was extracted by Merts and Schwarz[13-14] from brewer’s yeast. It can reverse the impaired glucose tolerance of diabetic rats by increasing glucose transport in hepatocytes, adipocytes, and cardiomyocytes and reduces the elevated levels of lipid peroxidation products. We have investigated the effects of chromium-enriched brewer’s yeast on STZ-induced type I diabetes of rats, which could improve the rats’ glucose tolerance.

In present study a strain of chromium-rich brewer’s yeast Cr-Y was obtained by culturing the N-Y yeast in 3200 µg/ml chromium acetate acid and its optional culture condition of the N-Y and the Cr-Y was investigated. The Cr-Y yeast cells could grow in different concentration of chromium and might be a potential microbe to treat chromium pollution in environment.

MATERIAL AND METHODOLOGY

Material

The original yeast cell strain was *Saccharomyces cerevisiae* named N-Y and was preserved by our own laboratory, and its chromium-stressed strain named Cr-Y, were prepared by our lab either. All of the reagents used below were analytic pure from Beijing Biochemical Company.

The start medium for N-Y was YPD as follows: 1 % glucose, 1 % peptone and 1 % yeast abstracts for liquid culture (add 2 % agar if cultured in flat plate).

Methods

Monoclonization of the brewer’s yeast

The N-Y stored in our lab was cultured in liquid YPD medium and were diluted to different concentration and were spread on flat plate, then cultured at 28℃ until the monoclones occurred in the flat plate. The separated monoclone was selected and transferred to a new flat plate. That procedure was repeated three times.

Inhibition of different concentration chromium on N-Y

N-Y cells were diluted to 10^5 /ml, and 100 µl were inoculated to flat plate with different content of chromium acetic acid as 0, 400, 800, 1600, 3200, 6400 and 12800 µg /ml. The result was observed after being cultured 3 days.

Optional Culture conditions for N-Y

Optimal carbon and nitrogen source

1 % glucose, sucrose, starch, maltose, lactose and dextrine was used as optional carbon material. After being cultured 24h, the N-Y fermentation liquid was detected by UV-sight spectro photometer at 600nm. The inoculum size was 10^5 cells /ml medium.

1 % beef extract, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, thiourea, peptone and NH_4Cl, was used as optional nitrogen material with the optimal carbon source. And then the other processes were as the selection of optimal carbon source.

Determine optimal medium by orthogonal method

Three factors and four levels orthogonal test was selected to determine the optimal medium for culturing the yeast cell. The three factors were glucose, $NaNO_3$ and yeast abstracts respectively. Each of the factors has four levels as 0.5%, 1%, 2% and 3%. The inoculum size was 10^5 cells /ml medium. After being cultured 24 h, the N-Y fermentation liquid was detected by UV-sight spectro photometer at 600 nm.

Optimal temperature, pH, rotation speed for N-Y cells

The monocloned N-Y cells were inoculated in fresh media and cultured at different temperature as 16℃, 20℃, 24℃, 28℃, 32℃, 36℃ and 40℃.

Then the N-Y cells were inoculated in fresh optimal media at the optimal temperature with different pH value as pH 3, 4, 5, 6, 7, 8, 9 and 10. The other steps were treated as the optimal temperature.

The N-Y cells were inoculated in fresh optimal media at the optimal temperature and pH with different rotation speed as 0, 50, 100, 150, 200 and 250 rotors per minute (rpm).

All of those above were treated as follows: 24 h later, the OD value of the fermentation liquid was detected by UV-sight spectrometry at 600 nm.

Preparation of chromium-enriched yeast Cr-Y

The N-Y cells were inoculated in fresh optimal media and cultured at the optimal conditions with different concentration of chromium acetic acid as 0, 400, 800, 1600, 3200, 6400 and 12800 μg /ml. Then the cells were subcultured to fresh optimal media with corresponding chromium acetate acid concentration after being cultured for 24 h and being transferred for at least 10 passages. The yeast cells were collected by centrifugation at 3000 rpm for 5 minutes, and then were washed by pure water (made by Millipore pure water instrument) 7 times until the chromium was removed completely which was identified by ICP-MS each time. The chromium concentrations and dried biomass of the Cr-Y and N-Y were detected by ICP-MS. The yeast with higher biomass and higher chromium named Cr-Y was used to investigate its optimal inoculation size and culturing time.

Detecting the content of chromium in the yeast by ICP-MS

The washed yeast was not dried until its weight did not changed at 85℃. Then the yeast was digested in HNO_3 and H_2O_2whose rate is $HNO_3 : H_2O_2 = 3 : 1$ solution with continuing heating at 200 ℃ for ICP-MS.

RESULTS AND DISCUSSION

Monoclonization of the N-Y

The N-Y cells were subcloned three times. The results showed that all of the clones were separated each other and the clones pointed by the arrows were selected for further culture and treatment (see Figure 1.).

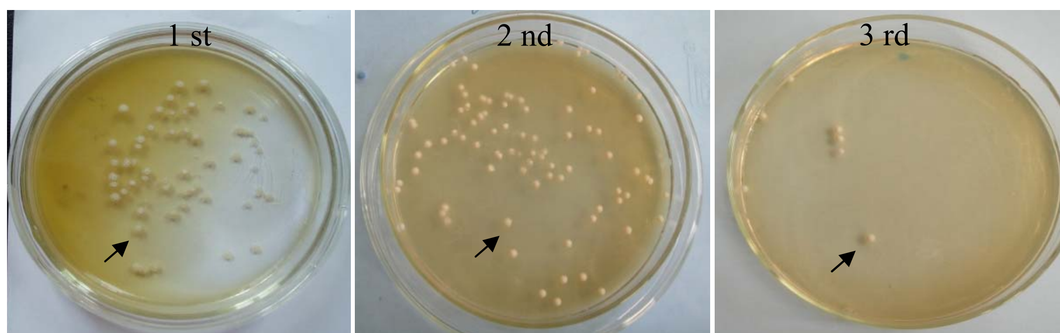


Figure 1 : Scheme of monoclonization results

Inhibition of chromium acetate concentration on yeast cells' growth

Different concentration of chromium acetate acid concentration on N-Y cells was different. Under low chromium acetate acid concentration (less than 3200 μg/ml), the inhibition effect was undefined. The N-Y cells was obviously inhibit when the chromium acetate acid concentration was 6400 μg/ml or higer (see Figure 2.). The data of other concentration of chromium acetic acid were not shown.

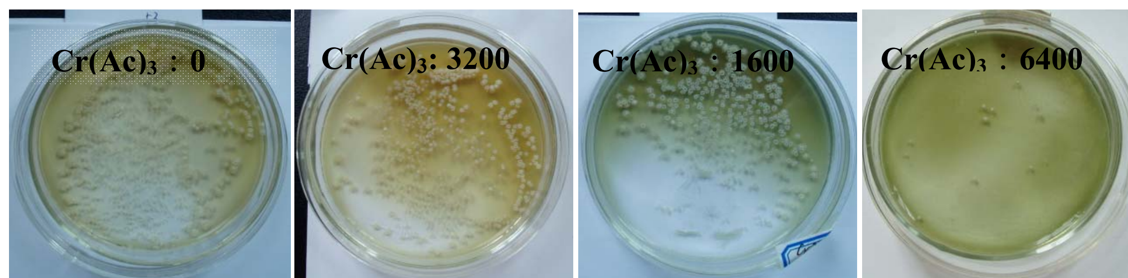


Figure 2 : Inhibition of different chromium acetate concentration on yeast cell growth

Optional culture conditions for n-y cells

The biomass of N-Y cells was effected by many factors such as carbon resources, nitrogen resources, yeast extracts, pH value, temperature, rotational speed etc. Effect of different sugars on N-Y growth was obvious. The N-Y cells could not utilize starch, and their capability of using lactose and dextine was poor. Glucose and sucrose could be utilized well, and the biomass of the N-Y was higer (see Figure 3.). The glucose was selected as the optimal carbon resource. The N-Y cells could utilize beef extract, NaNO3, NH4NO3, (NH4)2SO4, peptone, and NH4Cl, but the utilization of thiourea was poor (see Figure 4.). NaNO3 could enhance the biomass of the N-Y and was selected as the optimal nitrogen resource.

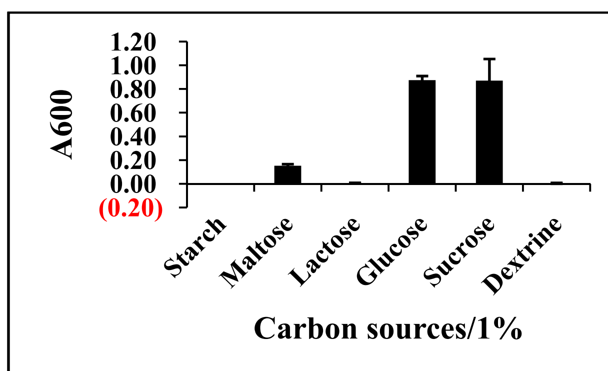


Figure 3 : Selection of optimal carbon resource

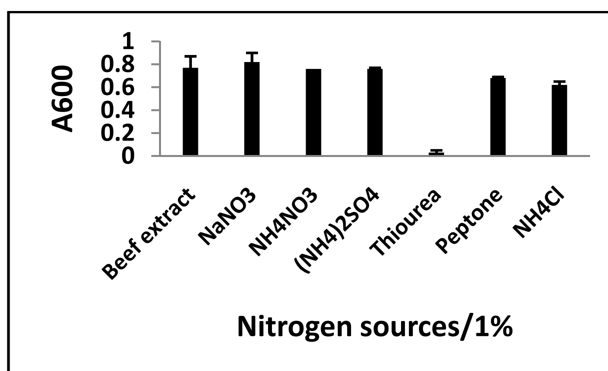


Figure 4 : Selection of optimal nitrogen resource

To determine the optimal medium for N-Y culturing, orthogonal method was used to identify the optimal concentration glucose, NaNO3 and yeast extract. Orthogonal experiment of three factors and four levels was designed to determine the optimal medium for N-Y (see TABLE 1.). The results of the orthogonal experiment showed that the optimal glucose, NaNO3 and yeast extracts concentration was as 3%, 0.5% and 2% (see TABLE 2.). To further determine the concentration of glucose and NaNO3, another two factors (glucose and NaNO3) and four levels (glucose: 2%, 3%, 4% and 5%; NaNO3: 0.125, 0.25, 0.5 and 1%) orthogonal experiment was designed. The result was the same as that of three factors and four levels (data not shown).

TABLE 1 : Orthogonal design of three factors and four levels

Glucose	$NaNO_3$	Yeast extract	OD
0.5	0.5	0.5	0.80
0.5	1	1	0.75
0.5	2	2	0.61
0.5	3	3	0.41
1	0.5	1	1.03
1	1	0.5	0.93
1	2	3	0.77
1	3	2	0.92
2	0.5	2	1.67
2	1	3	1.58
2	2	0.5	0.87
2	3	1	0.90
3	0.5	3	1.67
3	1	2	1.71
3	2	1	1.60
3	3	0.5	0.44

TABLE 2：Results of three factors and four levels orthogonal

	Glucose	$NaNO_3$	Yeast Abstract
X1	0.64	1.30	0.76
X2	0.88	1.24	1.07
X3	1.25	0.96	1.20
X4	1.36	0.64	1.11
range	0.28	0.56	0.26

When the temperature was less than 16 ℃, the N-Y cells could not grow, while the temperature was higher than 20°C, the cells could grow, and the biomass increased until 20°C. When the temperature is between 28 and 40°C, the biomass of the N-Y was relatively stable. So 28-36℃ was selected as optimal culturing temperature (see Figure 5.).

The effect of different initial pH on growth of N-Y cells was obvious. When the pH was less than 3 or higer than 11, the N-Y cells was hardly to grow. While the pH was between 4 and 9, the biomass of N-Y decreased with the increasing of pH. When pH was 4, the biomass was the highest. pH 4 was selected as optimal initial pH value.

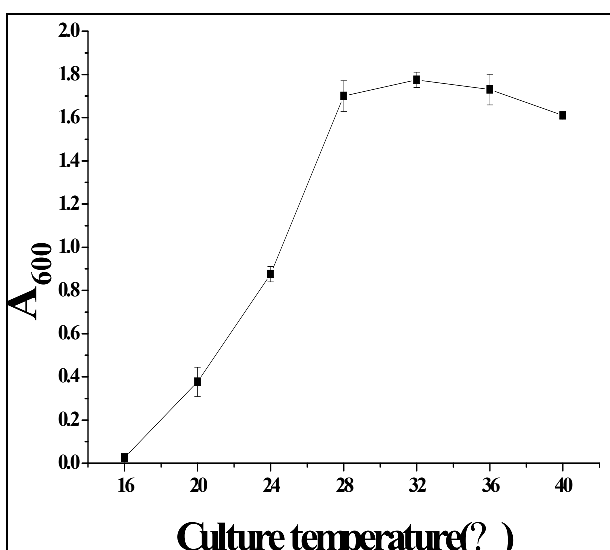


Figure 5 : Effect of temperature on N-Y cells

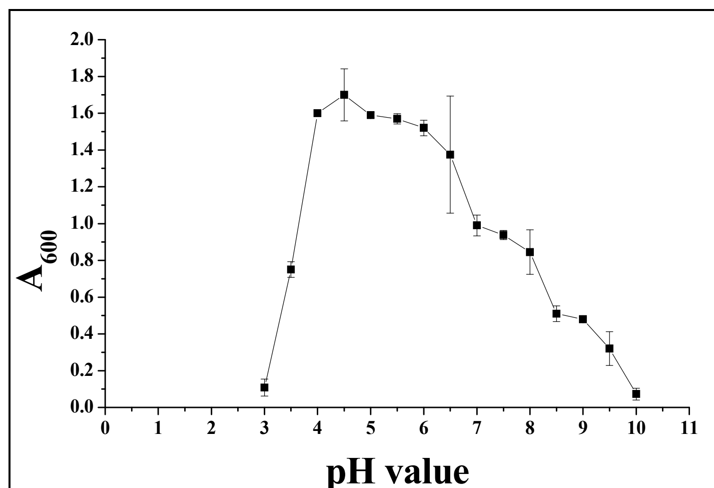


Figure 6 : Effect of pH value on N-Y cells

The effect of rotation speed of shaking bed between 0 and 250 rpm on biomass of the N-Y cells was investigated. The biomass of the N-Y would not increase with the increasing of the rotation speed until the speed was 200 rpm. That was to say when the rotation speed was 200 rpm or more, the biomass of the N-Y changed very tiny (see Figure 7).

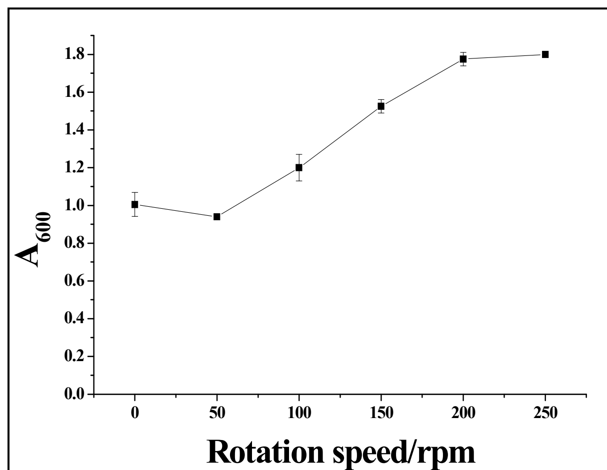


Figure 7 : Selection of optimal rotation speed for N-Y cells

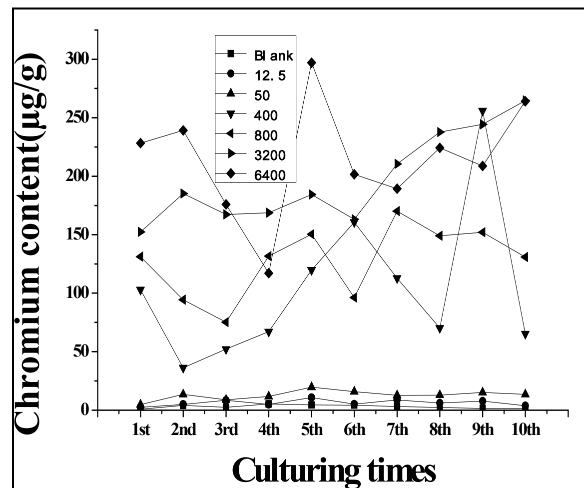


Figure 8 : Effect of different concentration of $Cr(AC)_3$ on different transferred passages

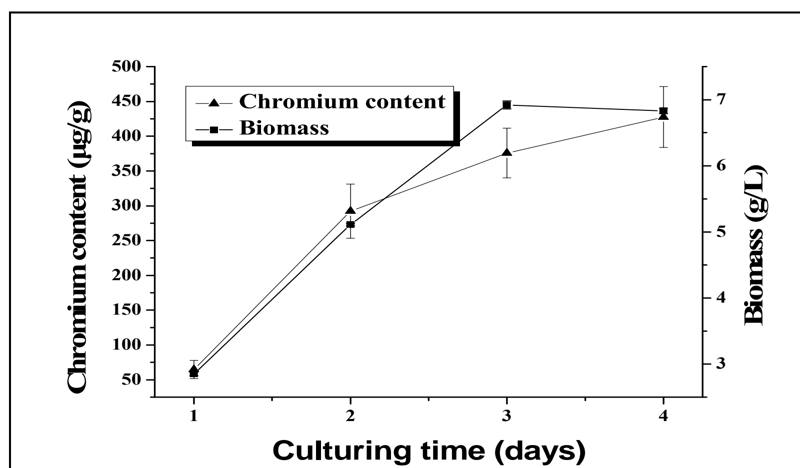


Figure 9 : Effects of culturing time on chromium content and Biomass of the Cr-Y

Preparation of chromium-enriched yeast (Cr-Y)

Different concentration of chromium acetate acid range from 0 to 6400 μg/ml was added to optimal media and was used to culture N-Y cells for 10 passsages. The results showed that the cells growing in 3200 μg/ml chromium acetate acid had higer chromium content and biomass (see Fig8.). After being transferred for 10 passages the cells grew in 3200 μg/ml chromium acetate acid could collect almost as high as that of those grew in 6400 μg/ml chromium acetate acid. So, 3200 μg/ml chromium acetate acid was selected as the optimal concentration added to the optimal media to culture the yeast for

another twenty passages. The content of chromium in Cr-Y was stable at about 450μg/g dried yeast and the biomass of the yeast was stable at 2.3 g/L after being transferred for 30 passages cultured for 24 h. So we got a strain of *Saccharomyces cerevisiae* and Cr-Y which could collect chromium from the media stably and was with stable genetic characteristics.

The chromium content and biomass in Cr-Y yeast after being cultured for different time, and the results showed that the optimal cultivation time was 3 days.

The inoculation size and culture time was selected by inoculating different volume of Cr-Y to fresh media and culturing for different days. The results showed that the optimal inoculation size was 1000 μl/100 ml, and the optimal cultivation time was 3 days (see TABLE 3). After being cultured for different time at the optimal conditions, the chromium content in N-Y yeast changed tiny, while that in Cr-Y yeast changed strongly.

TABLE 3 : Selecton of inoculation size and culturing time of Cr-Y

Inoculation size (μl/100 ml)	24 h (g/L)			48 h (g/L)			72 h (g/L)		
	N-Y	Cr-Y		N-Y	Cr-Y		N-Y	Cr-Y	
	Biomass*	Biomass*	Cr-C♦	Biomass*	Biomass	Cr-C♦	Biomass*	Biomass*	Cr-C♦
250	8.7	5.6	35.6	18	12.1	198.3	22.3	16.9	354.2
500	12.3	8.4	40.0	22.3	16.6	256.7	24.2	18.2	425.8
1000	16.7	10.5	38.7	25.7	20.3	375.1	24.6	20.5	503.7
1500	22.4	15.1	48.0	24.8	19.6	382.4	24.5	20.2	510.3

*** The unit for biomass is g/L; ♦ The unit for chromium content is μg/g.dried yeast.**

The Cr-Y yeast cells could grew in different concentration of chromium acetate acid and could collect chromium from the media and change the inorganic chromium to organic. The results suggested that the Cr-Y cell might be used to clear the chromium pollution in environment.

CONCLUSIONS

We got a strain of high-biomass chromium-rich *Saccharomyces cerevisiae* Cr-Y whose optimal culture condition was as: 30 g/L gucrose, 5 g /L $NaNO_4$, 20 g/L yeast extract, 28~40℃, pH4~6, rotation speed at 200 rpm or more than 200 rpm with 3200 μg/ml chromium acetate acid for different time and was transfered at least 30 passages. And the Cr-Y could collect chromium as 500 μg per gram dried yeast.

ACKNOWLEDGMENT

This paper is supported by 1) the Laboratory of Nuclear Analysis Techniques Fundation (K-123), Institute of High Energy Physics, Chinese Academy of Sciences; and 2) Anhui Provincial Microbiology Key Subject Project(KJ2012A249), NaturalScience Foundation of Anhui education department.

REFERENCES

[1] K.Schwarz, W.Mertz; A glucose tolerance factor and its differentiation from factor 3, Arch Biochem Biophys, **72(2)**, 515-8 **(1957)**.

[2] C.M.Davis, J.B.Vincent; Chromium oligopeptide activates insulin receptor tyrosine kinase activity, Biochemistry, **36(15)**, 4382-5 **(1997)**.

[3] U.A.Shindea, G.Sharma, Y.J.Xu, N.S.Dhalla, R.K.Goyal; Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus, J Trace.Elem.Med.Biol., **18(1)**, 23-32 **(2004)**.

[4] M.D.Althuis, N.E.Jordan, E.A.Ludington, J.T.Wittes; Glucose and insulin responses to dietary chromium supplements: a meta-analysis, Am.J.Clinical.Nutrition., **76(1)**, 148-155 **(2002)**.

[5] G.Y.Yeh, D.M.Eisenberg, T.J.Kaptchuk, S.P.Russell; Systematic review of herbs and dietary supplements for glycemic control in diabetes, Diabetes Care, **26(4)**, 1277-294 **(2003)**.

[6] R.A.Anderson et al.; Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes, Diabetes, **46(11)**, 1786-1791 **(1997)**.

[7] Agency revises chromium picolinate advice, Available from http://www.food.gov.uk/ news/newsarchive/2004/ dec/chromiumupdate, **(2004)**.

[8] Y.C.Li; Effects of brewer's yeast on glucose tolerance and serum lipids in Chinese adults, Biol.Trace.Elem.Res., **41(3)**, 341-7 **(1994)**.

[9] E.G.Offenbacher, C.J.Rinko, F.X.Pi-Sunyer; The effects of inorganic chromium and brewer's yeast on glucose tolerance, Plasma Lipids, and Plasma Chromium in Elderly Subjects, Am.J.Clinical Nutrition, **42(3)**, 454-461 **(1985)**.

[10] E.G.Offenbacher, F.X.Pi-Sunyer; Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects, Diabetes, **29(11)**, 919-925 **(1980)**.

[11] J.Racek, L.Trefil, D.Rajdl, V.Mudrova, D.Hunter, V.Senft; Influence of chromium-enriched yeast on blood glucose and insulin variables, Blood Lipids, and Markers of Oxidative Stress in Subjects with Type 2 Diabetes Mellitus, Biol.Trace.Elem.Res., **109(3)**, 215-30 **(2006)**.

[12] M.Machado, H.Soares, E.Soares; Removal of chromium, Copper, and Nickel from an Electroplating Effluent Using a Flocculent Brewer's Yeast Strain of Saccharomyces cerevisiae, Water, Air, Soil Pollution, **212(1)**, 199-204 **(2010)**.

[13] N.Mirsky, A.Weiss, Z.Dori; The effect of glucose tolerance factor in glucose uptake by yeast cells, Journal of Inorganic Biochemistry, **15(3)**, 275-9 **(1981)**.

[14] N.Mirsky, A.Aharoni, C.Rabinowitz, I.Izhaki; Naturally occuring chromium compound in brewer's yeast and the saltbush plant, J.Trace.Elem.Exp.Med., **12**, 111-124 **(1999)**.