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THE USE OF JERUSALEM ARTICHOKE FOR OBTAINING THE YEAST BIOMASS FOR FOOD AND FEED PURPOSES

SUMMARY

Researches on the use of Jerusalem artichoke tubers in the production of yeast biomass for food and feed purposes have been conducted. It was revealed that inulinase activity of yeast strains of *S. cerevisiae G* and *K. marxianus VKM Y*-1148 was 590 u / g and 150 u / g respectively at the cultivation in the inulin-containing medium. Strain *S. cerevisiae G* grows well in non-hydrolyzed juice of the Jerusalem artichoke obtained from fresh tubers. The juice of the Jerusalem artichoke has a high content of inulin (18.7%), phosphorus, potassium, B vitamins, biotin and can be used as a culture medium of yeast without mineral salts. The yield of biomass by culturing the yeast with aeration (30 °C, 24 h) was 60 g / L. The addition of $(NH_4)_2SO_4$ into the Jerusalem artichoke juice (0.6%) increased the accumulation of yeast biomass up to 74.6 g /L which is 24% higher of yeast biomass grown in beet molasses medium. The stimulating effect of the Jerusalem artichoke juice on *K. marxianus Y*-1148 during milk whey fermentation (30 ºC, with aeration) has been established. The maximum stimulating effect (2.8 times) is shown at the 15% content of Jerusalem artichoke in milk whey and during 24 h of incubation. The use of Jerusalem artichoke increases the yield of yeast biomass and enriches it with probiotic substances. It allows replacing the traditional molasses by the Jerusalem artichoke and to reduce the cost of production by increasing the yield of yeast biomass and by excluding mineral salts.

Keywords: *Helianthus tuberosus* L., inulin, yeast, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*.

INTRODUCTION

At present, highly effective methods of processing and utilization of plant raw materials are of great importance for solving food, energy and environmental problems. In this respect, an inulin-containing raw material, in particular Jerusalem artichoke (*Helianthus tuberosus* L.), is of great practical interest. The value of this plant is due to high yield, high-grade chemical composition, and ecological plasticity. Tubers Jerusalem artichoke is a rich source of carbohydrates, primarily fructosans, a significant part of which is inulin (73-86%). Inulin is a linear biopolymer consisting of fructose residues bound by β-1,2 bonds. At complete hydrolysis of inulin exposed inulinase (2,l-β-D-

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fructanfructanohydrolase) produced 95% fructose, 5% glucose, which are utilized by microorganisms (Yu et al., 2010). The tubers of Jerusalem artichoke contain significant amounts of macro- and microelements, nitrogenous substances, vitamins, including biotin, necessary for the development of microorganisms. Therefore Jerusalem artichoke can be considered as an alternative source of carbohydrates for biotechnology productions.

Microorganisms of different taxonomic groups can synthesize inulinases: bacteria, actinomycetes, microscopic fungi, yeast (Chi Z. et al., 2009). Yeast *Kluyveromyces marxianus* are well-studied inulinase producers, and inulinases strains are used to produce fuel ethanol from inulin-containing raw materials, which allows to unite the processes of saccharification and fermentation of inulin (Yuan W et al., 2008a). Today there are a limited number of producer strains of inulinases among Saccharomyces cerevisiae, insufficient studies on the use of inulin-containing raw materials for the production of *S. cerevisiae* biomass.

In this connection, studies have been carried out on the application of Jerusalem artichoke tubers to obtain biomass of yeast *S. cerevisiae* and *K. marxianus*. These yeasts have GRAS status, are able to quickly accumulate biomass with high protein, which is balanced with amino acid composition. The biomass of these yeasts can be used for the production of the food ingredients and feed.

MATERIAL AND METHODS

The tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) have been grown in the vicinity of Voronezh (Russia) and stored at 4-5 °C until use.

Microorganisms. As producers of biomass were used strain of baking yeast *Saccharomyces cerevisiae G* and a strain of *Kluyveromyces marxianus VKM Y*-1148. The inulinaseaktivity strain *Saccharomyces cerevisiae G* was obtained by the selection method in our laboratory (Sokolenko G.G., Karpechenko N.A., 2013; Sokolenko G.G., Karpechenko N.A., 2015), strain *Kluyveromyces marxianus* VKM Y-1148 was taken from the Russian Collection of Microorganisms (VKM).

Jerusalem artichoke juice was obtained by pressing from fresh crushed tuber of Jerusalem artichoke, heated diluted with water 2 times and heated at 100 degrees for 1 minute. After cooling, the juice was clarified by filtration and sterilized. The resulting Jerusalem artichoke juice contained sufficient quantities of nutrients necessary for yeast propagation (%): total nitrogen - 0.06; Reducing sugars - 0.8; Inulin - 18.72; Phosphorus - 0.046; Potassium - 0.3; Calcium - 0.022; Water - 89.32; pH is 6.0.

To determine inulinase activity of yeast, they were grown on a medium of the following composition (g / L): Inulin - 20.0; $(NH_4)_2SO_4$ - 5.0; KH_2PO_4 - 0.85- K_2HPO_4 - 0.15; $MgSO_4$ - 0.5; NaCl - 0.1; $CaCl_2$ - 0.1; Yeast extract – 2.0; pH is 5.0.

The culture medium based on molasses had a composition (g / L molasses diluted 4 times): $(NH_4)_2SO_4$ - 3.0; $(NH_4)_2HPO_4$ - 2.0; KCL - 1.0; pH is 5.0.

Fermentation was carried out in Erlenmeyer flasks (250 ml) with 50 ml of medium which was inoculated with overnight culture of yeast to a concentration of 1.0×10^7 cells/ ml and incubated at 30 °C on a rotary shaker (200 rpm). Samples were taken to determine the activity of inulinase and biomass.

K. marxianus was grown on unlighted curd whey, containing mineral salts (g / L whey): $(NH_4)_2SO_4$ - 3.0; $NH_4)_2$ HPO_4 - 2.0; KCL - 0.5. Fermentation was carried out with an initial concentration of inoculum of 1% at 30 ° C and 200 rpm. After fermentation the culture broth was heated 85-90 °C during 30 min to denature serum proteins and centrifuged at 6000g for 10 minutes to determine the mass yeast protein sediment. Was determined that yeast cells were 50% yeast protein.

The method for determining inulinase activity is based on the determination of reducing substances released during hydrolysis of the substrate under the action of the enzyme inulinase. One unit of inulinase activity is the amount of enzyme which catalyzes the formation of 1 g of reducing substance per 1 minute under the experimental conditions (Sokolenko G.G., Karpechenko N.A., 2013).

RESULTS AND DISCUSSION

Previously, we established the stimulating effect of inulin on the growth and reproduction of stains *S. cerevisiae* and *K. marxianus* when they have been grown in wort and whey (Sokolenko G.G. et al, 2010). In the present study it was revealed that inulinase activity of strains *S. cerevisiae G* and *K. marxianus Y*-1148 was 590 u / g and 150 u / g respectively at the cultivation in the inulin-containing medium (2% inulin). Strain *S. cerevisiae G* grows well in non-hydrolyzed juice of the Jerusalem artichoke obtained from fresh tubers. It makes it possible to use non-hydrolyzed Jerusalem artichoke juice as a growing medium for yeast. Known methods of growing *S. cerevisiae* using Jerusalem artichoke tubers presupposes hydrolysis of inulin with mineral acids, enzymatic preparations or activation of inulinases of Jerusalem artichoke (Patent of Russia No. 2064503, Patent of Russia No. 2144084, Patent of Russia No. 2301832, Patent of Russia No. 23590350). The studies on the cultivation of *S. cerevisiae G* on Jerusalem artichoke juice without preliminary hydrolysis of inulin have been carried out. It is established that the *S. cerevisiae G* grows well on the non-hydrolyzed juice of Jerusalem artichoke with the initial pH value (6.0) without the addition of mineral salts. The maximum accumulation of biomass was 60 g / L (cultivation for 24 h with aeration at 30 °C), which corresponds accumulation when grown on a molasses medium. Jerusalem artichoke has a low nitrogen content, and the introduction of $(NH_4)_2SO_4$ increased the accumulation of biomass. Research has been carried out on the effect of juice pH and concentration $(NH_4)_2SO_4$ on the yield of yeast biomass (Figure 1).

It was shown that the maximum accumulation of yeast biomass (74.6 g / L) was at pH 6.0 and concentration (NH_4) $_2SO_4$ - 0.6%, which is 24% more than with molasses (60 g / L) (Patent of Russia No. 2570628).

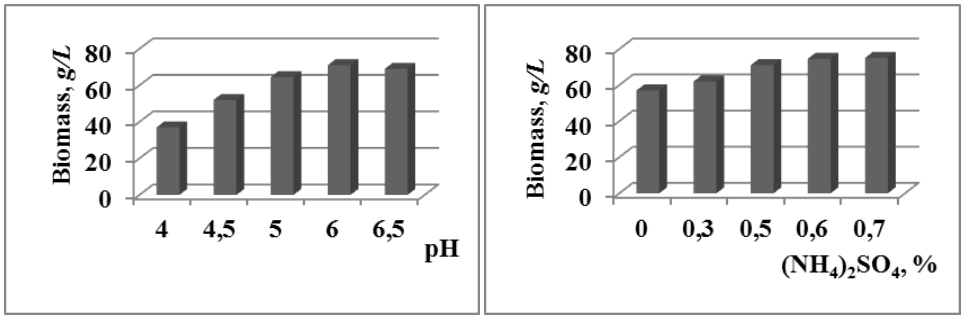

Figure 1. Effect of the pH of the Jerusalem artichoke juice and concentration $(NH_4)_2SO_4$ on the yield biomass of stain *S. cerevisiae G*.

The use of non-hydrolyzed Jerusalem artichoke juice allows replacing the traditional raw materials with the Jerusalem artichoke juice and reducing the production cost by increasing the yield of yeast biomass and excluding mineral salts.

Strain *K. marxianus VKM Y*-1148 synthesizes inulinase and β-galactosidase enzymes, is able to assimilate inulin and lactose in aerobic conditions and actively accumulate biomass (Fonseca GG et al, 2008). Therefore, it can be used in biotech production with using whey and inulin-containing plant material as substrates.

Studies have been conducted on the bioconversion of whey using *K. marxianus VKM Y*-1148 in yeast protein. Fermentation was carried out unlighted whey with aeration under batch cultivation conditions. It was revealed that as a result of the bioconversion in the whey the protein content increased 2.5 times, fat content increased 30 times, the product is similar to skim milk. The balance of essential amino acids was close to that of the "ideal protein". This allows us to recommend a yeast protein product for use for food and fodder purposes.

The juice of Jerusalem artichoke was added to the whey enriched with mineral salts, inoculated with an overnight yeast culture (1%), and fermented under aeration of the medium at 30 °C for 72 h.

It was revealed that the Jerusalem artichoke juice stimulates the growth and multiplication of yeast *K. marxianus VKM Y*-1148. The content in the serum of 15% of Jerusalem artichoke juice after 6 h of incubation increased the weight of yeast protein in comparison with the control in 2.4 times, after 8 h - in 2.5 times, after 24 h - in 2.8 times.

The influence of the concentration of Jerusalem artichoke juice and cultivation time on the efficiency of bioconversion of whey has been studied. Yeast was grown in whey with Jerusalem artichoke content 10%, 15%, 20% for 72 h, every 24 h in samples of culture liquid, the mass of the yeast protein precipitate was determined. The results of the studies are shown in Figure 2.

It is shown that during the fermentation of whey by *K. marxianus VKM Y*-1148 the Jerusalem artichoke juice stimulates the yeast and increases the yield of the bioconversion product.

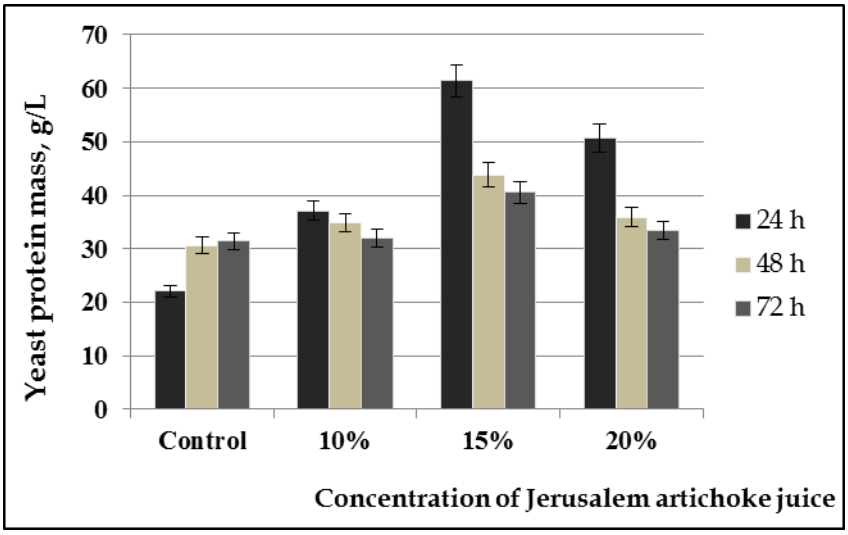

Fig.2. Effect of concentration juice of artichoke and cultivation time *K. marxianus VKM Y*-1148 on yeast protein mass

The maximum stimulating effect was at a content of juice 15% and the duration of cultivation for 24 h (61.4 g / L), which exceeded the control value (22.1 g / L) in 2.8 times, after 48 and 72 h the stimulating effect decreased to 43% and 29% respectively. At the same time Jerusalem artichoke juice enriches whey with biologically active substances (Group B vitamins, biotin, inulin, macro- and microelements) and increases the biological value of the bioconversion product.

CONCLUSIONS

As a result of the conducted studies it has been shown that juice from fresh Jerusalem artichoke tubers can be used to obtain the biomass of strains *of S. cerevisiae G* and *K. marxianus Y*-1148. K. The use of Jerusalem artichoke juice has a stimulating effect on *K. marxianus* and *S. cerevisiae*. It increases the yield of yeast biomass and enriches it with important biologically active substances. It allows replacing the traditional molasses by the Jerusalem artichoke and by reducing the cost of production by increasing the yield of yeast biomass and by excluding the mineral salts.

REFERENCES

Chi Z.T. Zhang G. Liu and L. Yue (2009). Inulinase-expressing microorganisms and applications of inulinases. Appl. Microbiol. Biotechnol., 82: 211-220.

Fonseca GG, Heinzle E, Wittmann C, Gombert A.K. (2008). The yeast Kluyveromyces marxianus and its biotechnological potential. Appl. Microbiol. Biotechnol. 79 (3): 339-354.

Sokolenko G.G., Karpechenko N.A. (2013). A novel Saccharomyces cerevisiae G strain possessing inuliase activity and good baking characteristics. Biotechnology in Russia, №6: 18-22.

Sokolenko G.G., Karpechenko N.A. (2015). Expression of inulinase genes in the yeasts Saccharomyces cerevisiae and Kluyveromyces marxianus. Microbiology (Mikrobiologiya), 84. № 1: 23-27.

Sokolenko G.G., Yarovoy S.A., Polyanskii K.K. (2010). Use of inulin-containing bioadditive during fermentation of whey or brewers wort by Saccharomyces cerevisiae or Kluyveromyces marxianus yeast. Biotechnology in Russia, №6: 42-46.

Patent of Russia No. 2064503, 1996.

Patent of Russia No. 44084, 2000.

Patent of Russia No. 2301832, 2007.

Patent of Russia No. 2359035, 2009.

Patent of Russia No. 2570628, 2015.

Yu J., Jiang J., Zhang Y., Lu H., Li Y., Liu J. (2010). Simultaneous saccharification and fermentation of Jerusalem artichoke tubers to ethanol with an inulinase-hyperproducing yeast Kluyveromyces cicerisporus. Chin. J. Biotechnol., 26: 982-990.

Yuan WJ, Ren JG, Zhao XQ, Bai FW (2008a). One-step ethanol fermentation with Kluyveromyces marxianus YX01 from Jerusalem artichoke. Chin. J. Biotech., 24(11): 1931-1936.