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WHEY FERMENTATION BY YEAST STRAINS *KLUYVEROMYCES MARXIANUS* UCM Y-2096 AND UCM Y-2388

Two Kluyveromyces marxianus strains have been tested for their ability to ferment lactose in the medium containing cheese whey powder (CWP) and lactose at different concentrations. The ethanol production by the selected strains was inhibited by the increase in substrate concentration (100 g/l). The strain K. marxianus UCM Y-2096 was found to yield the higher ethanol formation and to be more resistant to ethanol under microaerophilic conditions.

Key words: yeasts, ethanol, whey

It is estimated that the world production of whey is over 160 million tones per year. Whey is the main by-product of cheese production and exhibits a high biological (BOD) and chemical (COD) oxygen demand. It contains most of milk soluble components, e.g. lactose (4-5%), nitrogen compounds, proteins, lipids, vitamins. Cheese whey could pose a serious environmental problem due to high organic content. For this reason it should be utilized, however according to some reports more than 50 % of total whey produced in the world is discharged into the environment [6, 13].

One of the attractive ways to recycle cheese whey is to use it as a substrate for ethanol production. The conversion of liquid whey to ethanol is not economically competitive due to comparatively low lactose content. However the use of cheese whey powder (CWP) could overcome the shortcomings of such alcoholic fermentation. There are few studies on ethanol production from CWP where high ethanol yields are reported [3, 7].

Previously yeast strains from Ukrainian Collection of Microorganisms were screened for lactose fermentation [2]. Two strains *Kluyveromyces marxianus* UCM Y-2096 and 2388 were among the most active lactose-fermenting yeasts. The aim of this research is to determine the effect of inoculum size and substrate concentration on lactose fermentation by the given strains and determine their tolerance to the end product of alcoholic fermentation – ethanol.

Materials and Methods. Yeast cultivation was performed in the medium S1 and S2. The medium S1 was composed of (g/L): $(NH_4)_2SO_4$ – 3, $MgSO_4$ – 0.5, KH_2PO_4 – 1, yeast extract – 3. Lactose as a sole carbon source was added at concentrations 30-100 g/L. The medium S2 was prepared using CWP as follows: cheese whey powder was added to distilled water at 40 g/l concentration, pH was adjusted to 5.0, the solution was autoclaved to coagulate whey proteins, filtered through filter paper and sterilized. The mineral components and yeast extract at concentrations indicated above were added. 500 ml flasks containing 450 ml of cultivation medium were used. Flasks were fitted with rubber stoppers to prevent oxygen access. Experiments were carried out at 30 ^{0}C under stationary conditions (in the incubator). The yeast culture was grown in the medium S1 on a gyratory shaker at 30 ^{0}C for 18-20 h and used for inoculation at 1 % (v/v) and 10 % (v/v).

Cell growth was determined by measuring the sample optical density using the photoelectrocolorimeter KFK-2 at 540 nm. The standard curve (cell dry weight vs. optical density) was established.

Total reducing sugar concentration was determined using dinitrosalicylic acid (DNS) method [9]. Briefly, yeast suspension was centrifuged at 6000g for 5 min, 3 ml of DNS reagent (dinitrosalicylic acid – 1 g , phenol – 200 mg, sodium sulfite – 500 mg, sodium hydroxide – 1 g, distilled water – 100 ml) was added to 3 ml of appropriately diluted sample. The mixture was heated in the boiling bath for 5 min and 1 ml 40% sodium and potassium tartrate was added. Absorbance was measured at 540 nm. Sugar concentration was calculated based on the standard curve.

Ethanol concentration was measured by gas chromatography using “Chrom-5” chromatograph with flame ionization detector. Helium was used as the carrier gas, 80 ^{0}C. Flow rate was 20 ml/min.

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Results and Discussion. The effect of inoculum size on lactose fermentation and ethanol production by two lactose-fermenting yeast strains *K. marxianus* UCM Y-2096 and 2388 has been studied. The yeast cultures were cultivated in the medium S1 containing 30g/l lactose and in the medium S2 containing 40g/l cheese whey powder. Lactose assimilation by the studied strains significantly increased in the medium S1, when inoculum size was increased from 1 % (v/v) to 10 % (v/v) (Fig. 1). The addition of 1 % and 10 % inoculum of *K. marxianus* Y-2096 resulted in 67.54 % and 94 % lactose assimilation, respectively, during first 24 h cultivation, the complete substrate utilization was achieved after 48 h cultivation when 10 % inoculum was used while the same occurred after 72 h cultivation when 1 % inoculum was added. The similar trend was observed for *K. marxianus* Y-2388. However inoculum size did not affect ethanol production by *K. marxianus* Y-2096 – 16.4-16.7 g/l (Fig. 2). The 10 % inoculum addition resulted in the 73.5 % increase in ethanol production by *K. marxianus* Y-2388.

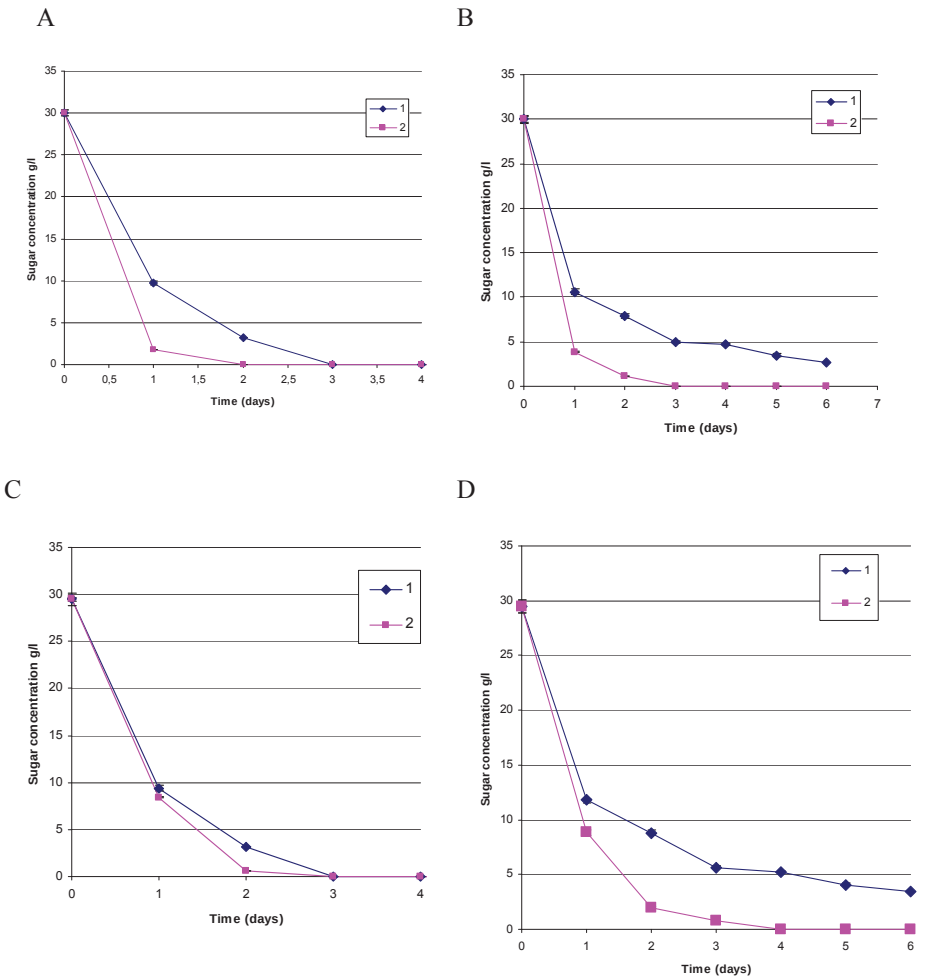

Fig. 1. The effect of inoculum size on lactose assimilation by *K. marxianus* UCM Y-2096 (A, C) and 2388 (B, D) in the medium S1 (A-B) and medium S2 (C-D). 1 – 1 % inoculum (v/v), 2 – 10 % inoculum (v/v)

As in addition to lactose whey contains proteins, lipids, mineral salts and other compounds which could affect yeast growth and lactose fermentation the ability of the studied strains to ferment lactose was studied in the medium S2 containing 40g/l whey powder (29-30 g/l lactose) (Fig. 1). The addition of 10% inoculum of *K. marxianus* Y-2388 resulted in the complete lactose utilization after 4 days cultivation, while the addition of 1% inoculum did not lead to the complete lactose fermentation even after 6 days cultivation. The increase in inoculum size did not significantly affect lactose utilization by *K. marxianus* Y-2096.

The addition of 10 % inoculum resulted in 12 % increase in ethanol production by *K.marxianus* Y-2096 compared to 1 % inoculum – from 13.9 to 15.61 g/l ethanol and in 64.6% increase in ethanol production by *K. marxianus* Y-2388 – from 8.29 to 13.65 g/l ethanol. Therefore 10% (v/v) inoculum was used in all the other experiments.

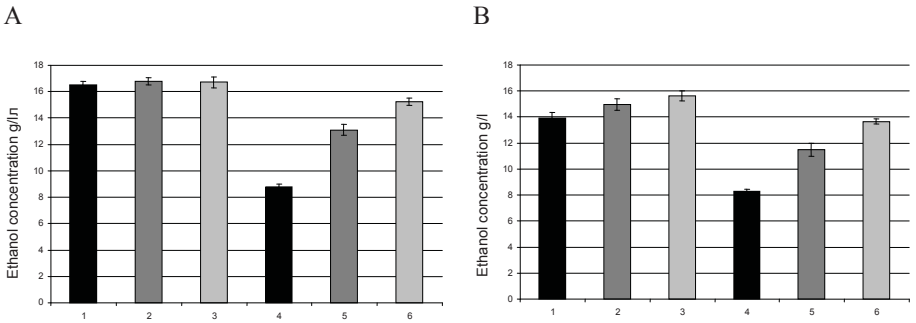

Fig. 2. The effect of inoculum size on ethanol production by *K. marxianus* UCM Y-2096 (1-3) and 2388 (4-6) in the medium S1 (A) and S2 (B). – 1 % inoculum (v/v), – 5 % inoculum (v/v), - 10 % inoculum (v/v)

Substrate concentration is one of the key parameters which determine the efficiency of fermentation process [6, 10]. The effect of substrate concentration on lactose fermentation by *K. marxianus* Y-2096 and Y-2388 has been studied by cultivating yeasts in the medium S1 containing 30 and 100 g/l lactose and the medium S2 containing 40 g/l whey (29-30 g/l lactose) and 70 g/l lactose (Fig.3, Table 1).

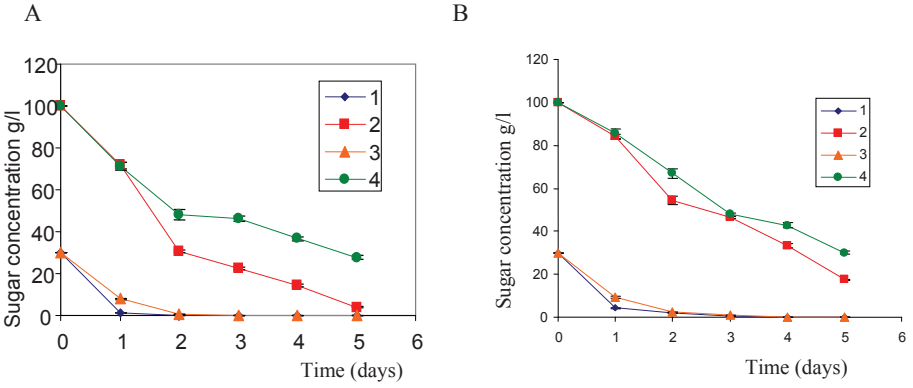

Fig. 3. The effect of substrate concentration on lactose assimilation by *K.marxianus* strains Y-2096 (A) and Y-2388 (B) in the medium S1 (♦, ■) and S2 (▲, ●). 1, 3 – lactose concentration 30 g/l; 2, 4 – lactose concentration 100 g/l

Table 1

Ethanol production by *K.marxianus* strains Y-2096 and Y-2388 at different lactose concentrations

Parameter	Yeast strain							
	K.marxianus Y-2096				*K.marxianus* Y-2388			
	medium							
	S1		S2		S1		S2	
	30 g/l lactose	100 g/l lactose	30 g/l lactose	100 g/l lactose	30 g/l lactose	100 g/l lactose	30 g/l lactose	100 g/l lactose
Final ethanol (g/l)	16.07	48.63	15.25	30.64	15.18	34.66	13.06	26.62
$Y_{P/S}$ (g ethanol / g lactose)	0.53	0.51	0.51	0.42	0.51	0.42	0.43	0.38
$Y_{\%T}$ (%theoretical yield)	98.15	93.78	94.11	78.55	93.7	77.72	80.63	70.55

Lactose fermentation by both studied strains was more efficient in the medium S1 than in the medium S2. The strain *K.marxianus* Y-2096 utilized 100% substrate after 48–72 h fermentation in the media containing 30 g/l lactose. When substrate concentration was increased up to 100 g/l the given strain fermented 94 % lactose in the medium S1 and 72.22 % lactose in the medium S2 after 5 days fermentation. The strain *K.marxianus* UCM Y-2388 was less efficient at fermenting lactose – 82.58 % substrate and 69.88 % substrate were utilized in the medium S1 and S2, respectively, after 5 days fermentation when the initial lactose concentration was 100 g/l.

The strain *K.marxianus* Y-2096 produced 15.25-16.07 g/l ethanol or 94-98% theoretical yield when substrate concentration was 30 g/l. After lactose concentration was increased up to 100 g/l ethanol production by both yeast strains decreased. The strain *K.marxianus* Y-2388 produced 13.06-15.18 g/l ethanol (80.63-93.7 % theoretical yield) at lactose concentration 30 g/l and 26.62-30.64 g/l ethanol (70.55–77.72 % theoretical yield) at lactose concentration 100 g/l. The similar inhibition of ethanol production by *Kluyveromyces* strains was observed by several researchers [4, 14]. Ozmihci et Kargi reported the decrease in ethanol production by *Kluyveromyces marxianus* DSMZ-7239 at substrate concentration higher than 75 g/l [10]. However Silveira et al. detected the opposite – the increase in substrate concentration led to the increase in ethanol yield obtaining near theoretical yields at lactose concentration higher than 50 g/l [12].

It should be noted that the studied yeast strains fermented lactose more actively in the synthetic medium S1 than in the medium S2 based on whey powder. The decrease in ethanol production by yeasts from whey may be attributed to their sensitivity to whey components, e.g., mineral salts or their osmosensitivity [5].

Besides the ability to produce ethanol at high yields the yeast strains must be tolerant to the elevated concentrations of the end product of alcoholic fermentation – ethanol. The ability of yeast strains *K. marxianus* Y-2096 and 2388 to tolerate ethanol at concentrations 2, 4 and 6 % (v/v) was studied under microaerophilic conditions in the medium S1. The addition of 2 % ethanol to the medium resulted in a slight inhibition of lactose fermentation and biomass yield and the strain *K. marxianus* Y-2388 was more sensitive to ethanol than *K. marxianus* Y-2096 (Fig. 4-5). The addition of 4-6 % ethanol to the medium led to the more pronounced inhibition of both lactose assimilation and cell growth. After 72 h cultivation the strains *K.marxianus* Y-2096 and Y-2388 assimilated 48.9 and 27.1% lactose present in the medium respectively under such conditions, while complete lactose utilization was achieved in the control (no ethanol added).

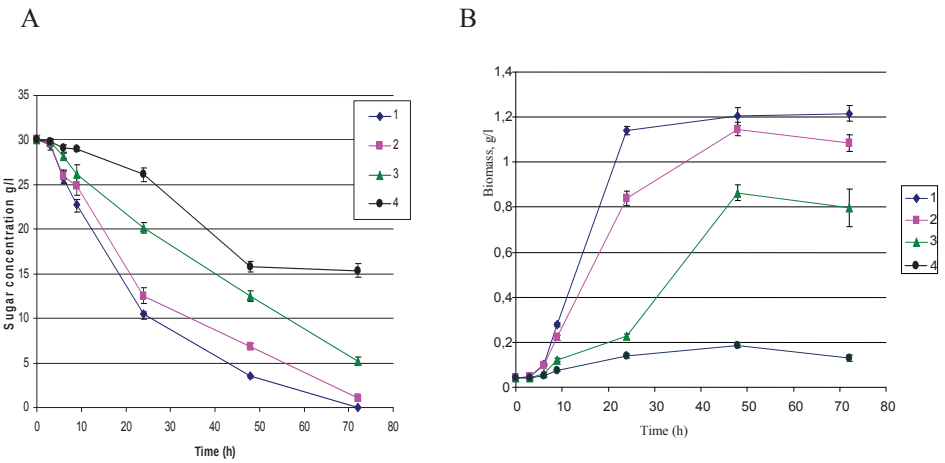

Fig. 4. Ethanol effect on lactose utilization (A) and growth (B) of the yeast strain *K. marxianus* Y-2096. 1 - control (no ethanol added), 2 – 2 % ethanol, 3 – 4 % ethanol, 4 – 6 % ethanol

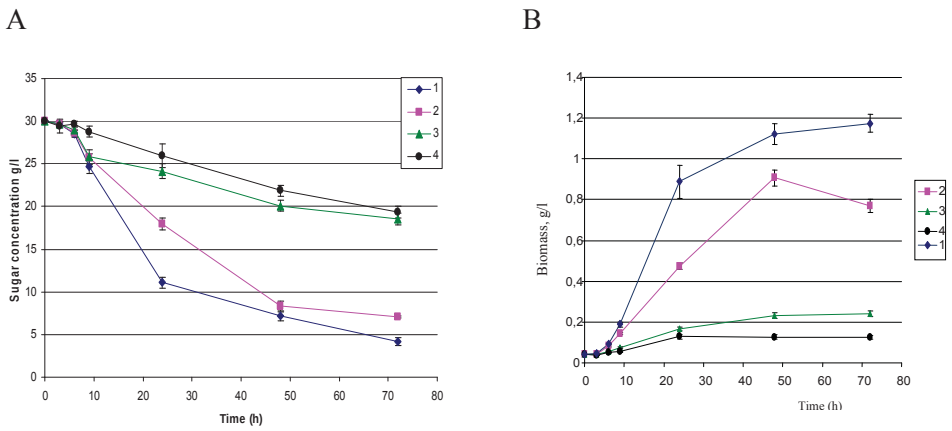

Fig. 5. Ethanol effect on lactose utilization (A) and growth (B) of the yeast strain *K. marxianus* Y-2388. 1- control (no ethanol added), 2 –2 % ethanol, 3 – 4 % ethanol, 4 – 6 % ethanol

The yeasts belonging to the genus *Kluyveromyces* do not possess high ethanol tolerance especially compared to other yeasts widely used in the fermentation industry – saccharomycetes [8, 11]. Golubev et al. reported that 4 % ethanol resulted in 40% decrease in the biomass yield while the addition of 6 % ethanol led to the 70-85 % growth suppression [1].

Thus it has been shown that the strain *K. marxianus* Y-2096 was less sensitive to ethanol and had higher ethanol production compared to the strain *K. marxianus* Y-2388. This strain was selected as a suitable candidate for ethanol production from lactose.

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ФЕРМЕНТАЦІЯ СИРОВАТКИ ШТАМАМИ ДРІЖДЖІВ *KLUYVEROMYCES MARXIANUS* УКМ Y-2096 ТА УКМ Y-2388

Р е з ю м е

Була досліджена здатність двох штамів дріжджів *Kluyveromyces marxianus* ферментувати лактозу в середовищі, що містило суху молочну сироватку та лактозу в різних концентраціях. Продукування етанолу даними штамами пригнічувалося при підвищенні концентрації субстрату (100 г/л). Штам *K.marxianus* УКМ Y-2096 був більш стійким до дії етанолу в мікроаерофільних умовах та був більш ефективним продуцентом етанолу.

К л ю ч о в і с л о в а: дріжджі, етанол, сироватка.

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ФЕРМЕНТАЦИЯ СЫВОРОТКИ ШТАММАМИ ДРОЖЖЕЙ *KLUYVEROMYCES MARXIANUS* УКМ Y-2096 И УКМ Y-2388

Р е з ю м е

Была исследована способность двух штаммов дрожжей *Kluyveromyces marxianus* сбраживать лактозу в среде, содержащей сухую молочную сыворотку и лактозу в различных концентрациях. Синтез этанола данными штаммами снижался при повышении концентрации субстрата (100 г/л). Штамм *K.marxianus* УКМ Y-2096 был более устойчив к действию этанола в микроаэрофильных условиях и был более эффективным продуцентом этанола.

К л ю ч е в ы е с л о в а: дрожжи, этанол, сыворотка.

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