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Study of some yeast strains in order to be used for ethanol production from whey

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Abstract

Lactose is one of the main components of cheese-whey and represents an interesting carbon source for the production of ethanol by whey waste by alcoholic fermentation.

The main objective of this study was to isolate and selection of some yeast strains able for ethanol production from cheese-whey in order obtain a good yield of substrate bioconversion. The strain coded KV3, belong to the genus *Kuyveromyces* has a good adaptability on the whey as substrate and has a high fermentative potential. In tested fermentative condition the obtained yield of fermentation of lactose into ethanol was of 70.33%.

Keywords: waste whey biovalorisation, *Kluyveromyces* spp., ethanol production

1. Introduction

The fermentation of lactose from whey to ethanol, using selected yeasts, has been frequently referred in the literature, since at least the 1940s [1].

Ethanol is produced through the fermentation of different by-products such as sugarcane, corn and wheat, sugar beet, cassava, cheese-whey among others.

Whey is the basic liquid by-product remaining after the precipitation and removal of milk casein during cheese manufacturing. The chemical composition of whey is dependent upon chemical composition of the milk, which varies with stage of lactation, feeding, breeding, individual animal differences, and climate. Few studies have shown that the whey protein composition of these milks follow the general lactation pattern. In addition, whey composition varies according to slight changes in milk processing parameters [2].

In Romania during the 2011 year about 40.000 tons of cheese was produced. About 30.000 tons of cheese is obtained from cow's milk resulting in approximately 250 million liters of whey. In the south of the country about 100 million liters of whey were produced and represent a serious problem for the dairy industries and for environmental protection.

The conversion of the lactose in cheese whey into ethanol is very competitive with the currently established processes, using different by-products such as sugarcane, corn and wheat, sugar beet, cassava or lignocellulosic biomass as raw material [1]. Obtained ethanol is a safe product, and can be used in food and beverages, pharmaceutical and cosmetic industries.

Yeasts able to ferment lactose are rather rare and include selected strains of *Kluyveromyces lactis, Kluyveromyces marxianus* and *Candida pseudotropicalis*.

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In the present study, some yeast strains for ethanol production from cheese-whey were isolated, characterized and selected for their ability to metabolise the lactose.

2. Materials and Method

2.1. Whey as substrates. Sweet whey was provided from S.C. Lacta S.A, Giurgiu, Romania. The physical and chemical characterisation of whey was realised by using Lactostar MILKLAB (U.K.) (Table 1).

Table 1. *Physical and chemical characteristics of sweet whey*

Whey characteristics	U.M.	Value
Lactose	%	4.94
Proteins	%	0.88
Lipides	%	0.06
Lactic acid	%	0.12
Ash	%	0.52
pH		6.7

2.2. Yeasts isolation. Isolation of yeasts was performed from sweet and acid whey, as well as from the appropriate dilutions, by using scarifying and spreading methods using the malt agar as culture medium [3]. The inoculated medium was then incubated for 5 days at temperature of 28 ^{o}C. After single colonies development, the pure cultures were performed by cultivation on slant agar medium and then were studied in order to morphological characterization.

2.3. Yeasts morphological characterization. Morphological characterization of isolated yeasts was done by macroscopically and microscopically analysis. The biomass from pure cultures was inoculated punctiform on the malt agar medium in Petri dishes and then incubated for 10-12 days at temperature of 28 ^{o}C and was finally obtained specific colonies. Colonial characteristics were evaluated as well as: perimeter, color, shine, the relative amount of biomass and colony aspect.

The growth characteristics on liquid medium were also analyzed. The cells were inoculated in malt extract broth and then incubated for 2-3 days at temperature of 28 ^{o}C. After incubation the turbidity and possible presence of the foam and the sediment aspect were noted.

Using stock pure cultures the microscopic preparations were made and then studied microscopically (microscope Optika, Germany). Cell shape and size of cells, particularities of the yeast budding, the presence of ascospores were noticed.

2.4. Yeasts biochemical characterization. Physiological-biochemical characterization was performed taking into account that yeast may metabolize a characteristic spectrum of carbon sources. The ability of yeasts to assimilate glucides found in sweet whey was studied by using modified Beijerink method [4].

Depending on the ability to metabolize the glucides tested, yeasts growth and multiplicate with different rates. For this experiment a synthetic medium deficient in carbon source, solidified with agar and enrichment with teste glucides was used. A suspension with concentration varying from 10^4 to 10^5 cells was prepared to be use as inoculum. Each Petri dish is divided into six sectors. This suspension was used to uniform spread on the surface of the solidified medium on Petri dishes. After drying, in each sector are drawn grooves with a sterile loop crystalline powder charged with carbohydrate test. After incubation for 5-6 days at temperature of 26 ^{o}C, the development of biomass around carbohydrate test by sector was observed. It is considered that yeast assimilate tested sugar when biomass formed covers about1/3 of the area for delimited sector.

2.5. Alcohol tolerance. Alcohol tolerance test was done as follows: culture medium was prepared with the following composition (g/L): $(NH_4)_2SO_4$ 0.1; KH_2PO_4 0.1; $MgSO_4 \cdot 7H_2O$ 0.01; ethylic alcohol 3 ml; distilled water 100 ml, pH = 7.2. The culture medium was distributed into sterilized tubes at temperature of 120 ^{0}C for 15 min. After cooling the inoculation was done by loop with yeast inoculum. Then, it was incubating for 72 hours at temperature of 25^{0}C. Test is considered positive if increase the turbidity of the culture medium is appearing and then sediment formation is present.

2.6. Urease test. Urease test is a confirmatory test for anascogene yeast species and is based on the differential ability of yeasts to hydrolyze high

concentrations of urea from complex medium containing peptone as organic nitrogen source [4]. The results are assessed on the basis of color intensity of the culture medium that changes from yellow orange to pink purple.

2.7. Fermentative potential characterization of isolated yeasts. Fermentative potential characterization of isolated yeasts was performed by studying the dynamics of alcoholic fermentation of lactose by quantifying the CO_2 emissions measured at different time intervals [5].

The fermentative medium had the following composition (g/L): lactose 2; $(NH_4)_2SO_4$ 0.5; KH_2PO_4 0.1; $MgSO_4 \cdot 7H_2O$ 0.005; yeast extract; pH = 6.0.

Using the general equation of alcoholic fermentation taking into account the total amount of formed CO_2, the amount of alcohol formed (A), the amount of sugar fermented (Z_i), and alcohol yield (η_{C2H5OH}) were calculated.

3. Results and Discussion

Six yeast strains were isolated from whey and were characterized morphologically. The main characteristics are presented in Table 2.

Table 2. Morphological characteristics of the yeast isolates

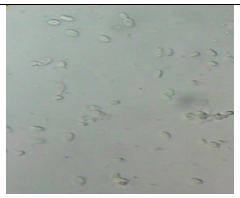
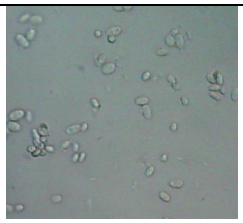
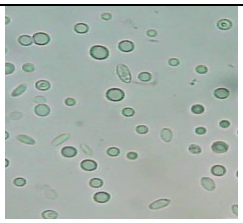
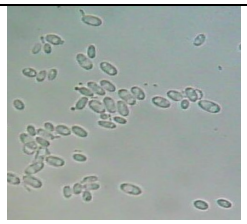
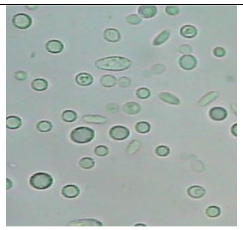
Yeast strain code	Macroscopic characteristics	Microscopic aspect	
KV2	Glossy colonies with cream-white colour with full edge and convex profile, sticky consistency. Colony diameter = 3.6 mm	Ovoid and spherical cells, with no forming filaments. Unipolar bud. Singular and grouped cells.	
KV3	Confluent colonies, with shine surface and light yellow, sticky consistency. Colony diameter = 2.2 mm	Ovoid cells with filamentous elements. Linear and branched filaments.	
KV4	White and shiny colonies, adherent to the medium, sticky consistency. Colony diameter = 3.4 mm	Oval cells with various sizes. Isolated or grouped in small clusters.	
KV5	Confluent glossy colonies with yellowish-gray colour, sticky consistency. Colony diameter = 2.6 mm	Spherical and oval cells. Isolated or grouped in small clusters.	

Table 2. (continued)

Yeast strain code	Macroscopic characteristics	Microscopic aspect	
KV6	Mate colonies, adherent to the medium with white-gray colour, sticky consistency. Colony diameter = 3.6 mm	Oval cells without filaments. Unipolar bud. Isolated or grouped in small clusters.	
KV7	Glossy colonies, yellowish white colour, adherent to the medium, sticky consistency. Colony diameter = 3.8 mm	Spherical globular and oval cells. Unipolar bud. Singular cells.	

The results of the test for selected yeasts to use the alcohol as the single carbon source and urease production are presented in Tabel 3.

Table 3. The abilities of selected yeasts to use the alcohol as the single carbon source and to produce urease

Yeasts strain	Ethanol use as the single carbon source*	Urease production**
KV 2	+	+ + +
KV 3	-	+ + -
KV 4	+	+ + -
KV 5	-	+ - -
KV 6	-	+ + +
KV 7	-	+ + -

* + positiv reaction; - negative reaction ** +++ intense reaction; ++- mean reaction; + - - low reaction; - - - absent reaction

As can be observed in Table 3 the ability of tested yeasts to use alcohol as carbon source was reached for strains coded KV2 and KV4. This test makes these yeast strains to be able for lactose fermentation processes in order to obtain ethanol. These properties are important for strains able to produce ethanol to be alcohol resistant and do not uses it as a carbon source is required because this reduces the ethanol yield.

Regarding the urease test all isolated yeast strains were able to produce urease. Table 3 shown that two strains coded KV2 and KV6 present an intense urease activity (ascospors producers), three of them coded KV3, KV4 and KV7 presented a medium urease activity and one strain coded KV5 present low urease activity it is an anascogen yeast.

The results about isolated yeast strains ability to metabolize the different simple glucides are presented in Table 4.

As can be seen in Table 4, three of the strains coded KV3, KV5 and KV6 were able to metabolise glucose, five of them KV2, KV3, KV5, KV6 and KV7 were able to metabolise sucrose as carbon source. All of isolated yeast strains presented the ability to metabolize lactose.

Table 4. The simple glucides spectrum metabolise ability of isolated yeasts

Glucides	Yeast strains					
	KV 2	KV 3	KV 4	KV 5	KV 6	KV 7
Glucose	-	+	-	+	+	-
Saccharose	+	+	-	+	+	+
Lactose	+	+	+	+	+	+
Xilose	-	-	-	-	+	+
Maltose	+	-	+	-	+	+
Fructose	+	-	-	+	+	+

In accordance to the dichotomics keys for yeast taxonomy [6] the strains KV3 and KV6 belong to the genus *Kluyveromyces*.

All yeasts which had the ability to ferment lactose were further tested for alcoholic fermentation dynamic evaluation. The inoculum in concentration of 10^5 CFU/mL was inoculated in fermentative medium containing 2% lactose as the single carbon source in Erlenmeyer flasks. The flasks were weighted daily to observe the fermentation rate, by CO_2 forming evaluation. The dynamics of fermentation yield are presented in Figure 1.

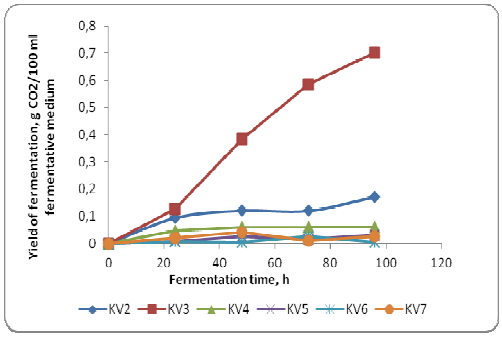


Figure 1. The lactose fermentation dynamics by selected yeast strains

As it can be observed from Figure 1, yeast coded KV3 is performant, it produce the highest CO_2 amount of 0.7 g/100 mL by lactose alcoholic fermentation. Also, all yeasts except for KV3 strain, presented lower fermentation yields, its liberated small CO_2 amounts, varying between 0.024 and 0.072 g/100 mL and. The yeast coded KV3 was selected for further experiments regarding the lactose from whey fermentation.

The ethanol content of the samples was calculated using the general equation of alcoholic fermentation [7]. The results obtained are presented in Figure 2.

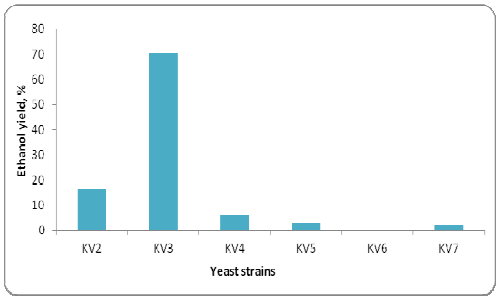


Figure 2. Alcoholic lactose fermentation abilities of yeast isolates from whey

The yeast coded KV3, presented the good ability to ferment lactose. The estimated fermentation parameters were: the amount of fermented sugar (Z_i) was 1,4 g % lactose and the ethanol yield was 70.33%.

4. Conclusion

Six yeast strains were isolated from whey and further characterized and tested for their ability to ferment lactose, in order to biovalorisation of whey to ethanol production.

All of the isolated yeast strains presented the ability to ferment fructose. Based on morphological analysis and some biochemical characteristic evaluation the strains were classified as belong to the genus *Kluyveromyces*.

The yeasts were then tested based their abilities to ferment lactose. The strain coded KV3 presented the good ability to produce ethanol from lactose. This strain will be tested to ferment whey and by optimization the fermentative conditions to produce ethanol by valorization cheese whey; an important dairy industry by-product considered to be an important pollutant for the environment.

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