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preliminary communication

Galactose-Fermenting Yeasts as Fermentation Microorganisms in Traditional Koumiss

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Summary

Yeast flora composition was determined in 94 samples of traditional Central Asian koumiss. The dominant yeast was Saccharomyces unisporus which, though lactose non-fermenting, ferments galactose well and is mainly responsible for alcoholic fermentation of koumiss.

Sacch. unisporus normally causes slower and less clean alcoholic fermentation than Sacch. cerevisiae since it produces larger amounts of minor fermentation compounds such as glycerol, succinic and acetic acid. It has low alcohol producing capacity and cannot complete grape must fermentation. It is more vigorous only in milk whey where it achieves clean alcoholic fermentation.

Keywords: koumiss, yeast, *Saccharomyces unisporus*

Introduction

Koumiss is a traditional beverage produced in Central Asia derived from the lactic and alcoholic fermentation of mare's milk *(1)*. The yeast producing its alcoholic fermentation are lactose-fermenting species from the *Kluyveromyces* genus and, more often, non-lactose fermenting strains belonging to *Saccharomyces* genus *(2–5)*. The species found most frequently is *Sacch. unisporus*, a yeast that ferments only lactose and galactose vigorously.

This paper reports on the microbiological (yeast) studies carried out on a large number of koumiss samples collected in Kazakhstan from various environmental conditions (desert, steppe, high mountain). Fermentation tests were carried out in different media with *Saccharomyces unisporus*, i.e. the dominant yeast, to assess the technical fermentation properties.

Material and Methods

The samples of koumiss were taken in Kazakhstan from nomad people. They were collected in 50 mL sterile jars and stored at 8–12 °C until the analyses. Isolation was on Sabouraud Dextrose Agar (Oxoid, Basingstoke RG24 OPW, UK). After incubation at 25 °C for 5 days sporigenous *Saccharomyces* and *Kluyveromyces* yeast strains were identified and classified respectively according to Yarrow (1984) and van der Walt and Johannsen (1984) *(6,7)*.

The technological and fermentative properties of *Sacch. unisporus* were determined using 28 strains from the collection of the Department of Protection and Improvement of Food Production (University of Bologna) with *Sacch. cerevisiae* strain 6167 as the control (from the collection of the same department). Three nutrition media were used: (1) Yeast Nitrogen Base (Difco, Detroit, MI 48232, USA) with added glucose at an 8% w/v concentration; (2) residue acid milk whey from Parmigiano-Reggiano cheese processing with 5% w/v added galactose; (3) grape must. The fermentation was in 100 mL bottles with 5 mL of mineral oil in non-shaken culture. The sugar content and fermentation products (ethanol, glycerol, succinic acid, acetic acid, malic acid) were determined enzymatically using specific Boehringer kits (Boehringer, D-68298 Mannheim 31, Germany).

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Results and Discussion

The composition of the yeast flora for the 94 samples of koumiss (Table 1) showed a major presence of non-lactose fermenting yeasts. Among these, *Saccharomyces unisporus* was the dominant yeast in 68% of the samples examined and was the most frequent in samples from isolated and higher mountain areas.

Sacch. unisporus is a yeast whose taxonomic position has been known for quite some time *(8)*. It forms asci with usually just one spore, and its fermenting action, though vigorous, is confined to glucose and galactose.

Sacch. unisporus has been found in a number of dairy milk products *(5,9–15)*. Its dominant position in koumiss can be explained by the behaviour of the different lactic bacteria that ferment lactose well using just glucose and not galactose from the disaccharide *(16,17)*. The galactose secreted in the medium can be fermented by the non-lactose fermenting yeasts *(18)*.

The behaviour of *Sacch. unisporus* strains determined on a synthetic medium and acid milk whey highlighted considerable differences from strain to strain (Figs. 1 and 2 show the extreme behaviour of the strains), but they frequently started immediate and fast fermentation and were more vigorous than the control strain *Sacch. cerevisiae* 6167. In wine must (Fig. 3), on the other hand, *Sacch. unisporus* produced less immediate, slower and incomplete fermentation. It should be noted that there was still some gas production when fermentation was halted after 14 days.

The amount of minor alcoholic fermentation compounds produced was very much medium-specific. In the synthetic medium (Table 2), *Sacch. unisporus* caused less clean fermentation than *Sacch. cerevisiae*: larger amounts of glycerol and acetic acid were produced, but succinic acid was at a similar level in spite of considerable strain-specific variance.

In milk whey (Table 3), *Sacch. unisporus* also caused clean alcoholic fermentation with minor fermentation compound levels similar to *Sacch. cerevisiae*. Only succinic acid was found at higher than average levels, while acetic acid varied considerably.

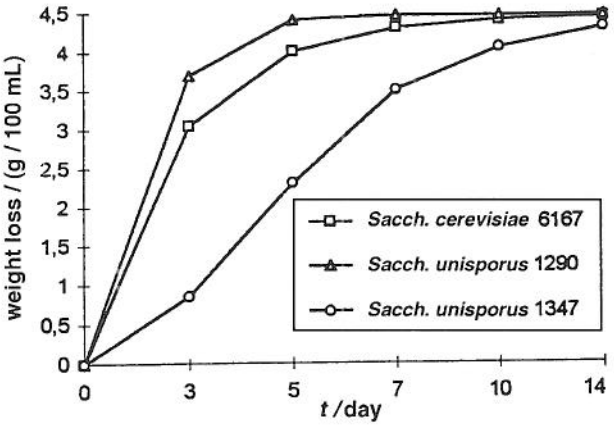

Fig. 1. Syntetic medium fermentation curves

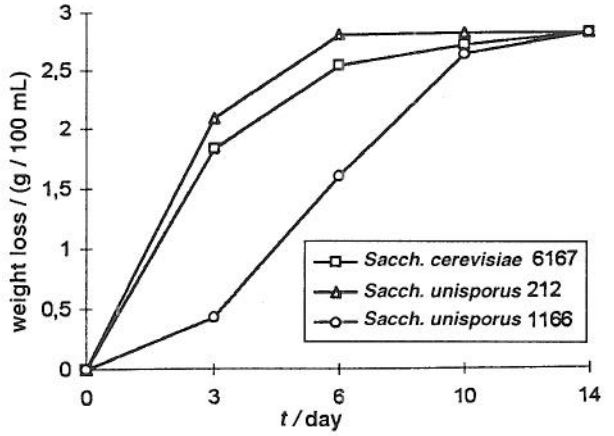

Fig. 2. Milk whey fermentation curves

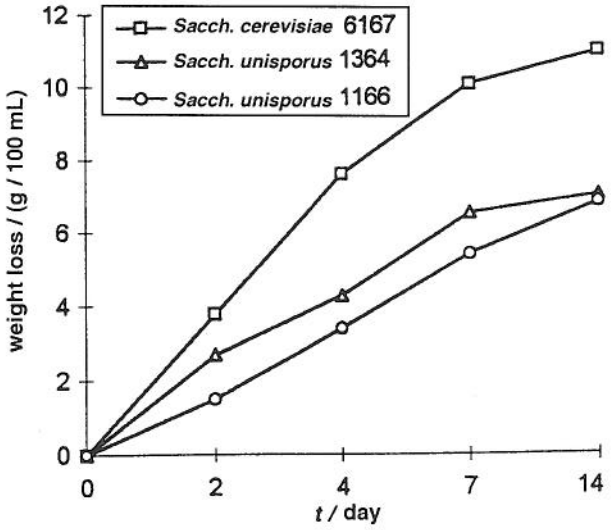

Fig. 3. Grape must fermentation curves

Table 1. Characteristics of yeast cultures isolated from 94 samples of koumiss

Yeast	Species	Strain isolated	Dominant in samples*
Non-fermenting	not identified	165	13
Lactose-fermenting	*Kluyveromyces marxianus* var. *lactis*	6	2
	Kluyveromyces marxianus var. *marxianus*	6	2
Fermenting galactose but not lactose	*Saccharomyces unisporus*	192	64
	Saccharomyces cerevisiae physiological race *cerevisiae*	18	6
	Saccharomyces cerevisiae physiological race *steineri*	12	4
	Candida buinensis	18	3

*Dominant species is >80%

Table 2. Fermentation products by *Saccharomyces unisporus* in synthetic medium

Product	*Saccharomyces unisporus* (28 strains)			*Saccharomyces cerevisiae* n. 6167
	Min.	Mean	Max.	
Ethanol (volume fraction)	34.0	43.8	46.2	46.1
Glycerol*	3.49	4.73	5.87	2.78
Succinic acid*	0.05	0.15	0.48	0.21
Acetic acid*	0.32	0.58	0.72	0.40

*Amounts expressed as grams per litre

Table 3. Fermentation products by *Saccharomyces unisporus* in milk whey + galactose, at pH = 3.2

Product	Whey specifications	*Saccharomyces unisporus* (28 strains)			*Saccharomyces cerevisiae* n. 6167
		Min.	Mean	Max.	
Lactose (g/L)	3.5		unchanged		unchanged
Galactose*	5.07		absent		absent
Lactic acid*	1.395		unchanged		unchanged
Ethanol (volume fraction)		2.16	2.50	2.91	2.73
Glycerol*		1.32	1.88	3.05	2.21
Succinic acid*		0.39	0.58	0.67	0.66
Acetic acid*		0.05	0.19	0.32	0.16

*Amounts expressed as grams per litre. Galactose added.

Table 4. Fermentation products by *Saccharomyces unisporus* in grape must

Product	Must characteristics	*Saccharomyces unisporus* (28 strains)			*Saccharomyces cerevisiae* n. 6167
		Min.	Mean	Max.	
pH	3.20	3.12	3.15	3.22	3.18
Sugar*	170	15	23	30	<1
Ethanol (volume fraction)		5.10	6.27	7.90	11.06
Glycerol*		6.09	7.70	9.53	3.97
Succinic acid*		0.49	0.80	1.20	0.66
Acetic acid*		0.05	0.10	0.17	0.11
Malic acid*	2.58	2.22	3.07	4.54	2.30

*Amounts expressed as grams per litre

In grape must (Table 4), *Sacch. unisporus* produced abnormal amounts of glycerol, while succinic acid was around double that of *Sacch. cerevisiae*. The amount of acetic acid was very small. Malic acid, which usually decreases due to malo-alcoholic fermentation, showed a degree of increase. The ability to synthesise malic acid is rare in yeast but is well-known in some *Saccharomyces* strains *(19)*. Ethanol production was lower than in *Sacch. cerevisiae*.

The properties described here, the inability to complete fermentation and non-alcohol tolerance make a yeast unsuitable from an oenological viewpoint. Nonetheless, its performance in grape must deserves a more in-depth treatment given the continued production of gas after 14 days, as mentioned previously, and the imbalance between residual sugar content and minor fermentation compounds that do not, however, compensate for lower ethanol production.

Conclusion

The technical and fermentative properties of *Sacch. unisporus* explain why this yeast is found in cheese making by-products (it is, in fact, the main alcoholic fermentation agent in koumiss) though it does not perform the same function in other media with a sugar content such as grape must. It is non-lactose fermenting and ferments galactose well. It is normally absent in milk and its derivatives. This monosaccharide can be released by lactic bacteria. Thanks to its fermentation properties, it is not improbable that *Sacch. unisporus* could have some interesting applications in the production of beverages such as koumiss which have come to the attention of the dairy and cheese processing industry.

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Kvasci koji fermentiraju galaktozu kao mikroorganizmi vrenja za tradicionalni kumis

Sažetak

U 94 uzorka tradicionalnog kumisa iz srednje Azije određen je sastav flore kvasaca. Prevladavao je kvasac Saccharomyces unisporus koji, iako ne fermentira laktozu, dobro fermentira galaktozu te je najodgovorniji za alkoholno vrenje kumisa. Sacch. unisporus obično uzrokuje polaganije i manje čisto alkoholno vrenje nego Sacch. cerevisiae, jer nastaje veća količina nusproizvoda kao što su glicerol, jantarna i octena kiselina. On ima malu sposobnost proizvodnje alkohola i ne može dovršiti vrenje mošta. Samo u sirutki mlijeka postiže se čisto alkoholno vrenje.