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ISOLATION AND TAXONOMIC STUDY OF YEAST STRAINS FROM BULGARIAN DAIRY PRODUCTS

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Summary

Twelve yeast strains were isolated and determined taxonomically from traditional dairy products, in which they occurred as additional microflora. Morphological, cultural and physiological tests were carried out resulting in determination of the strains to species. The recent study aims at establishing the species variety of the contaminants in different Bulgarian dairy products, which caused disadvantages in them.

Introduction

Depending on the environmental conditions the microorganisms do not grow individually, but in close associations. The yeasts usually adapt to coexistence with lactic acid bacteria as the one of the group gain the upper hand over the other or the both groups grow intensively together. By this process specific interactions take place. The yeasts play favorable effect upon the bacteria due to the change in pH value in the medium and the secretion of biological active substances such as vitamins, enzymes, aminoacids etc. In some cases the yeasts significantly increase the viability of the lactic acid bacteria [2, 4]. The investigations of the microflora found in the dairy drinks kefir and kumis proved the presence of lactic acid bacteria and yeasts, as the latter play stimulating effect on the bacteria.

However, by other dairy products, the yeasts are undesirable additional microflora. As a common, the dairy products are especially favorable environment for growth of yeasts due to the acidic reaction of the medium. Another important condition for their development is their ability to grow at low temperatures and also lactose dissimilation. The basic yeast species, which decrease the quality of the dairy products, are mainly representatives of the genera *Candida* (*C. sphaerica*) *Debaryomyces*, *Mycoderma*, *Saccharomyces* (*S. dairensis. S. unisporus*) and *Rhodotorula* [6, 8, 10].

Some representatives of the genus *Rhodotorula* cause staining and give a bitter taste of the products. It was established that yeast species such as *C. sphaerica* ferment lactose, owing to gas formation in the dairy products. Their detrimental effect leads to preparing of non-quality products in the milk processing [3]. For example, from the fermented cream is hard to obtain a churned

butter. By the curd, which is a secondary product of the white and yellow cheese processing, the presence of yeasts leads to the so called "yeast taste". This reflects on the taste quality of the curd if these microorganisms exceed ten thousands per gram product. The yeasts from genus *Mycoderma* do not ferment carbohydrates, but oxidize the ethanol to acid and the lactic acid to carbon dioxide and water. Their growth on the surface of the soft cheeses and other products causes worsening of the quality.

For prevention of these and other disadvantages improvement of the general hygiene is required and the technological regime should be observed [9].

The aim of this study was the isolation and taxonomic characterization of the yeasts from various dairy products found in different sources and regions in Bulgaria, which occur to be main spoilage microorganisms in the traditional Bulgarian food products.

Materials and Methods

Isolation sources. By this investigations traditional Bulgarian dairy products were used: goat's and cow's yogurt from the Troyan region (the villages Prolesha and Tabashko) and Sofia region (the villages Paskal and Losen); cow's and goat's cheese from the same regions; curd, obtained by the production of yellow cheese in Sofia and Samokov and butter, made in Sofia region.

Isolation procedures. Routine microbiological procedures and selective nutrition media for isolation of yeasts were used. The most appropriate medium for this purpose was the modified Saborou medium with the following composition: dextrose 4 % (w/v), peptone 1 %, agar 2 %. pH was adjusted with 1 % HCl at 4.0 for griping the growth of the other microorganisms. After 72nd hour cultivation at 25-28^{O}C single, morphologically well-formed colonies were isolated. The appropriate ones were re-cultivated several times for purity.

Nutrition media and growth conditions. The cultivation was carried out in YPD (yeast extract-pepton-dextrose) and BA (beer agar) medium. For the taxonomic tests the yeasts were incubated at the optimal for every strain temperature.

Identification. The strains were identified according to the procedures described by Kreger van Rij, Barnett et al. and Kurtzman [1, 5, 7].

Morphological researches. The colonies were tested and described on morphological agar and yeast-malt agar (YM). The strains were also inoculated in YM broth for determination of their cultural characteristics (pellicle, sediment or ring formation). The sexual and asexual type of propagation was also studied.

Physiological investigations. Yeast nitrogen base (YNB) medium containing 1 % carbohydrate as a single carbon source was used for testing the assimilation of carbon sources by yeasts. The results were registered after the 3rd, 7th, 14th, 21st and the 28th day at the corresponding optimal growth temperature. Nitrates, nitrites, L-lysine, ethylamine, cadaverine, creatine were used for investigations of the assimilation of different nitrogen sources.

The ability of some carbohydrates for anaerobic assimilation (fermentation) was determined by using Durham glass tubes after 3 weeks reporting. The quantity of the tested carbohydrates was 2 %.

Additional tests. DBB test, production of extracellular starch compounds, urease test for hydrolyzation activity, acid production from glucose.

The isolated cultures were stored by freeze-drying with protecting medium Faibeech (sucrose 10 %, gelatine 1.5 % and agar 0.1 %).

Results and discussion

Twelve yeast strains were isolated, purified and further identified from different dairy products, made in our country. Differential tests were applied, including morphological and physiological characteristics, which facilitate the opportunity for identification of the yeasts. The set of these tests allow the information gathering for the studied objects and for determination of their systematic status to species.

The morphological data of the investigated strains are described and represented in Table 1. It got clear that teliospores and ballistospores were formed by none of them. Ascospores formed five of them, namely Ta, Pr, K_7, K_8, C_6. The most of the strains showed pseudomycelium formation. A part of the identified strains belonged to ascomycetes (order *Ascomycotina*) and the rest to order *Deuteromycotina* – Imperfect yeasts.

The physiological and biochemical researches of the yeasts strains were carried out, by using over 60 tests for assimilation of carbon and nitrogen sources. The results are shown in Table 2. The utilization of 17 carbohydrates, 2 polysaccharides, 2 glycosides, 10 alcohols, 5 organic acids and five nitrogen sources was tested. The most microorganisms utilized galactose, sucrose, ethanol, mannitol, glycerol, lactic acid. Except four of the strains, the lactose was assimilated as a single carbon source. The rest sugars, acids and alcohols were utilized to different extend. The assimilation of sugars by fermentation was also variable. Negative fermentation ability had only three of the cultures. It was weak by three of the strains and very vigorous was registered mainly by the representatives of genus *Kluyveromyces*.

The data from the taxonomic researches (colonial and cell morphology and physiological characteristics) were analyzed by using the Manuals of Kreger van Rij, Barnett et al. and Kurtzman [1, 5, 7]. The represented results allowed us to refer the tested cultures to the listed species: *Kluyveromyces marxianus*, syn. *Saccharomyces fragilis*, (Ta), *Kluyveromyces marxianus* var. *bulgaricus* (Pr), *Kluyveromyces marxianus* var. marxianus (C_6), *Kluyveromyces lactis* var. *lactis* (K_8), *Trichosporon beigelii* (K_1) *Debaryomyces hansenii* var. *hansenii* (K_7), *Candida famata*, which is the imperfect form *of Deb. hansenii* (K_9, I_5), *Candida sphaerica*, imperfect form of *Kl. lactis* (M), *Candida crusei*, anamorph of *Issatchenkia orientalis* (C_3), *Candida rugosa*, syn. *Mycoderma rugosa*, *Mycotorula rugosa* (K_{11}) and *Rhodotorula mucilaginosa* (Rr).

In the investigated dairy products, the identified yeast species were as follows: in cow's yogurt (Paskal village, Sofia region and Tabashko village, Troyan region) – *Kl. marxianus*; in goat's yogurt (Prolesha village, Troyan region) – *Trich. beigelii*, *Deb. hansenii* var. *hansenii*, *C. krusei*, *Kl. marxianus* var. *marxianus* and var. *bulgaricus*, *C. rugosa*; in curds (Sofia and Samokov region) – *C. famata* var. *famata* and *C. sphaerica*; in cow's and goat's cheese (Sofia and Troyan region) – *Deb. hansenii* var. *hansenii*, *Kl. lactis* var. *lactis*, *C. famata*, *C. sphaerica* and *C. famata* var. *famata*; in butter (Sofia region) – *Rhod. mucilaginosa* and *C. sphaerica*.

By some of the strains there were differences from the type cultures, for example K_1, K_{11}, which is probably due to the specific composition of their natural habitats. In the studied cheese samples, the yeast species were reported in the listed quantities: *Deb. hansenii* comprises 50 % from the tested microflora, *Kl. lactis* var. *lactis* was 40 %, *C. famata* 7-8 % and *C. sphaerica* – 2-3 %. In the tested curds samples the quantity of the strains was as follows: *C. famata* – 60 %, and *C. sphaerica* – 40 %. In the cow's yogurt were found mainly strains from the species *Kl. marxianus*. The highest diversity of species showed the samples from goat's yogurt:

Table 1. Morphological characteristics of yeast strains.

Characteristics	Strains											
	K_1	K_7	K_9	K_{11}	C_3	C_6	Ta	Pr	K_8	I_5	M	Rr
Surface	rough, granular	wrinkled, raspberry-like	rough, granular	wrinkled, verrucose	rough, acicular	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Margin	crispulate	undulating	undulating	undulating	crispulate	entire	crispulate	irregular	entire	entire	slightly crispulate	entire
Colour	cream, acicular	white, dull	white, acicular	white, semi-dull	cream-yellow	cream, dull	cream, dull	cream, semi-dull	cream, dull	cream-white	cream, dull	coral-red
Elevation	convex	slightly convex	convex	convex	convex	nearly flat	slightly convex	slightly convex	slightly convex	convex	semi-convex centre	semi-convex
Cells	ellipsoidal, filamentous	spheroidal to short ellipsoidal	spheroidal	ellipsoidal, filamentous, septate mycelium	ellipsoidal, cylindrical, filamentous	oval, ellipsoidal	oval, ellipsoidal ovoidal	oval, ellipsoidal	ellipsoidal	spheroidal, ellipsoidal	ellipsoidal, cylindrical	ellipsoidal, cylindrical
Ascospores	-	+	-	-	-	+	+	+	+	-	-	-
Ballistospores	-	-	-	-	-	-	-	-	-	-	-	-
Pseudo-mycelium	+	-	-	+	+	+	+	+	+	+	+	+
True mycelium	+	-	-	+	+	-	-	-	-	-	-	-
Growth at 30°C	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	+	-	-	+	+	+	+	+	+	-	+	+
Growth at 40°C	-	-	-	-	+	+	+	+	-	-	-	-
Survival (%)	1.32	40.0	2.40	0.11	2.60	9.42	23.50	10.58	1.90	17.64	14.80	98.7

Table 2. Physiological characteristics of yeast strains.

Assimilation	Strains											
	K_1	K_7	K_9	K_{11}	C_3	C_6	Ta	Pr	K_8	I_5	M	Rr
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	-	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	-	+	+	+	+	+	+	+
Maltose	-	+	+	-	-	-	-	-	+	+	-	+
Lactose	+	+	+	-	-	+	+	+	+	+	+	-
L-Sorbose	D	+	+	+	-	-	-	-	+	+	-	+
Cellobiose	D, W	+	+	-	-	+	D, W	D, W	+	+	+	+
Trehalose	-	+	+	-	-	-	-	-	D, W	+	+	+
Melibiose	-	-	+	-	-	-	-	-	-	D	-	-
Raffinose	+	+	+	-	-	+	+	+	+	+	+	+
Melezitose	-	+	+	-	-	-	-	-	+	+	+	+
Inulin	-	+	-	+	-	+	+	+	-	+	+	-
Starch	W	+	-	-	-	+	W	+	-	-	-	-
D-Xylose	-	+	D	-	D	+	+	+	D	+	+	+
L-Arabinose	-	+	+	-	-	+	+	+	-	+	-	+
D-Arabinose	-	-	D, W	+	-	-	-	-	-	D, W	-	+
D-Ribose	+	W	-	-	-	-	-	-	-	D, W	-	+
L-Rhamnose	-	W	+	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	-	-	+	+	+	+	+	ND	ND
α-Me-D-Glucoside	-	+	+	-	-	-	-	-	D	+	-	D
D-Glucosamine	+	+	D, W	-	-	-	-	-	-	-	-	-
Ethanol	+	+	+	+	+	+	+	+	+	D	+	-
Methanol	-	-	-	-	-	-	-	-	-	-	-	-
myo-Inositol	-	-	-	-	-	-	-	-	-	-	-	-
Erythritol	+	+	+	-	-	-	-	-	-	+	-	-
Ribitol	-	+	D	-	-	+	+	+	-	+	-	+
Galactitol	-	+	D, W	-	-	-	-	-	-	D	-	-
D-Mannitol	-	+	+	+	-	D	+	D	+	+	+	D, VW
D-Glucitol	+	+	+	+	-	+	+	+	+	+	+	
Xylitol	+	+	+	+	-	+	-	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+
DL-Lactate	+	+	-	+	+	+	+	+	+	+	+	-
Succinate	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	-	-	-	+	-	-	-	-	-	-	+
D-Gluconate	-	+	-	-	-	-	W	+	-	+		+
D-Glucuronate	+	-	-	-	-	-	-	-	-	-		-
Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
Nitrite	-	-	-	-	-	-	-	-	-	D, W	-	-
L-Lysine	+	+	+	+	+	+	+	+	+	D, W	+	-
Ethylamine	+	+	+	+	+	+	+	+	+	+	+	+
Cadaverine	+				+						+	-

Legend: delayed growth (D), weak growth (W), very weak growth (VW), no data (ND).

Table 2. (continued).

Fermentation	Strains											
	K_1	K_7	K_9	K_{11}	C_3	C_6	Ta	Pr	K_8	I_5	M	Rr
D-Glucose	-	D, W	+	-	+	+	+	+	+	W	+	-
D-Galactose	-	VW	-	-	-	+	+	+	+	-	+	-
Sucrose	-	-	D, W	-	-	+	+	+	+	-	+	-
Maltose	-	VW	-	-	-	-	-	-	-	+	-	-
Lactose	-	-	-	-	-	+	+	+	+	-	+	-
Raffinose	-	D, W	D	-	-	+	+	+		-		-
Trehalose	-	+		-	-	-	-		-		-	-
α-Me-D-Glucoside	-	-	-	-	-		D		-	-	-	-
Inuline	-	-		-	-	+		+	-	-		-
DBB reaction	+	-	-	-	-	-	-	-	-	-	-	+
Starch formation	-	-	-	-	-	-	-	-	-	-	-	-
Urea hydrolysis	+	-	-	-	-	-	-	-	-	-	-	+
Acetic acid production	-	-	-	-	-	-	-	-	-	-	-	-
50 % Glucose	D	+	+	-	-	-	-	-	-	+		+
0.01 % Actidion		-			-	+			+			
w/o vitamins	-	+	W	-	+	-	-	+	-	+	-	+

Legend: delayed growth (D), weak growth (W), very weak growth (VW), no data (ND).

genera *Trichosporon* and *Kluyveromyces* about 30 %, the rest species (*D. hansenii*, *C. krusei*, *C. rugosa*) presented in equal quantities. In the butter the main contaminants were *Rhod. mucilaginosa* and *C. sphaerica*.

As a conclusion, the yeasts isolated from different Bulgarian dairy products belonged to five genera: *Kluyveromyces* (2 species), *Debaryomyces* (1), *Candida* (4), *Trichosporon* (1) and *Rhodotorula* (1).

References

1. Barnett, J. A., R. W. Payne, D. Yarrow, 1990. *Yeasts: Characteristics and identification*, Cambridge: University Press.
2. Chomakov, H., 1990. *Microbiological investigations of the milk and dairy products*, Sofia: Zemizdat, 65 (in Bulgarian).
3. Fleet, G. H., 1990. *J. Appl. Bacteriol.*, **68**, 199-211.
4. Higins, I., D. Best, D. Jones, 1988. *Biotechnology. Principles and applications*, Moskva: Mir, 93-102 (in Russian).
5. Kreger van Rij, N. J. W., 1987. *The Yeasts. A taxonomic study*, Amsterdam: Elsevier Science Publishers B. V.
6. Kurtzman, C. P., 1990. Classification and general properties of yeasts. In: The *yeasts. Biotechnology and bioanalysis*, H. Verachtert, R. De Mot (Ed), London: Academic Press, 184.
7. Kurtzman, C. P., J. W. Fell, 1998. *The Yeasts. A taxonomic study*, Amsterdam: Elsevier Science Publishers B. V.
8. Mossel, D. A., 1980. Experience with some methods for the enumeration and identification of yeasts occurring in foods. In: *Biology and activities of yeasts*, F. D. Skinner, S. M. Passinfre, R. K. Divenpart (Ed), London: Acad. Press, 279.
9. Robinson, R. K., A. Y. Tamine, 1981. Microbiology of fermented milks. In: *Dairy Microbiology,* vol. 2, London: Applied Sciences Publishers, 245- 278,
10. Wood, B. J. 1998. *Microbiology of fermented foods*, sec edn, vol. 1, London: Blackie Academic Professional, 96.

ИЗОЛИРАНЕ И ТАКСОНОМИЧНИ ПРОУЧВАНИЯ НА ЩАМОВЕ ДРОЖДИ В БЪЛГАРСКИ МЛЕЧНИ ПРОДУКТИ

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Резюме

Изолирани и определени таксономично са 12 щама дрожди от традиционни хранителни млечни продукти, в които те се явяват допълнителна микрофлора. Проведени са морфологични, културални и физиологични проучвания, в резултат на които щамовете са идентифицирани до вид. Целта е установяване на видовото разнообразие на замърсителите в различни български млечни продукти, предизвикващи недостатъци в тях.