Characterization of yeasts isolates originating from Hungarian dairy products using traditional and molecular methods

PhD thesis

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1. Introduction

Different yeast species can be isolated from dairy products. Except for the fermentation of kefyr and kumiss by adding a mixture of lactic acid bacteria and yeasts, they naturally occur in high number in several types of dairy products. Due to their excessive growth, certain yeast species may play an important role in the spoilage of dairy products. Typical defects caused by spoilage yeasts are gas production, yeasty flavour and other off-flavours, discolorations and changes of texture (Fleet, 1990; Viljoen and Greyling, 1995; Deák and Beauchat, 1996; Jakobsen and Narvhus, 1996).

In recent years, yeasts have been increasingly considered as important agents in the maturation process of some cheeses. First, by metabolizing lactic acid, they raise the pH thus facilitating the implantation of an acidosensitive bacterial biota. Second, by their proteolytic and lipolytic activities, they generate the precursors of aromas (free aminoacids and and fatty acids) that may contribute significantly to the final flavour (Romano et al., 1996). *Geotrichum candium* is known to be involved in cheese ripening by producing various sulphur compounds from different amino acid precursors that contribute to cheese variety (Berger et al., 1999; Demarigny et al, 2000).

Recognizing these two important sides of their activity, yeasts occuring in dairy products have created increasing interest all over the world. So far, however, only sporadic data exist on yeasts in Hungarian dairy products. For this reason a survey was initiated on yeasts in dairy products available in the Hungarian markets.

For the purposes of isolation and enumeration of yeasts from foods the use of media which allow the recovery of all kinds of yeast while inhibit bacterial growth and reduce fungal spreading is recommended (King et al., 1986; Fleet, 1990; Deák, 1991; Beuchat, 1993). For controlling growth of bacteria chloramphenicol, oxytetracycline, gentamycin or some other antibiotics appeared to be equally effective (Beckers et al., 1986).

Various attempts have been made to improve enumeration of yeasts in the presence of filamentous fungi by reducing the colony diameter of spreading molds. King et al. (1979) described a medium containing chloramphenicol for the inhibition of bacteria as well as dichloran and rose bengal to retard the spreading of molds.

For the purposes of enumerating and isolating yeasts from dairy products the best suitable medium has yet to be selected or developed. To this end, a comparative study was made to assess and statistically evaluate the performance of various mycological media. Eleven media were tested for their efficiency to support the growth of yeasts in the presence of molds and bacteria in samples of blue-veined cheese.

For the analysis of yeast microbiota the accurate identification of yeast isolates is essential. Identification and classification of yeasts are traditionally made by means of phenotypic methods to asses morphological, physiological and biochemical characteristics. However, considerable experience and skill is required in the performance and evaluation of some 60-90 specified test (Deák, 1995). Commercialy available identification kits offer several advantages over traditional methods, but they were designed only for clinically important yeasts. The simplified identification method (SIM) (Deák and Beuchat, 1987, 1993; Deák, 1991) on the other hand, is restricted to those yeast species that occur most frequently in foods. SIM relies on 30 tests only, and the results can be obtained within a week.

In the last years, a considerable development in the identification and classification of yeasts has come with the introduction of molecular techniques. Some of them have been recomended by numerous authors for rapid identification of yeast isolated from various foods, wine and beer. These include pulsed-field gel electrophoresis (PFGE, karyotyping) (Naumov et al, 1993; Tornai-Lehoczki and Dlauchy, 1996), restriction enzyme analysis (RFLP) (Meaden, 1990; Querol and Ramon, 1996), PCR-based techniques (ribotyping, RAPD analysis) (Baleiras Couto et al. 1994; 1995; Romano et al, 1996; Dlauchy et al, 1999; Prillinger et al, 1999;

Andrighetto et al, 2000; Deák et al, 2000) and sequencing of ribosomal RNAs (Cappa and Cocconelli, 2001). The other aim of my study was to check the suitability of SIM procedure, kariotyping, ribotyping and RAPD-, and microsatellite-PCR analysis for the rapid characterization of yeasts isolated from Hungarian dairy products and to assess their biodiversity. Intraspecific typing was made in order to differentiate strains according to their origin and to find correlation between the origin of yeasts and types of dairy products.

2. Materials and methods

Reference strains

Candida catenulata NCAIM Y1032*, Candida glabrata* CBS 138, *Candida lusitaniae* CBS 6936, *Candida maltosa* CBS 5611, *Candida mesenterica* NCAIM Y1072, *Candida parapsilopsis* CBS 604, *Candida rugosa* CBS 613, *Candida sake* CBS 159, *Cryptoccocus curvatus* NCAIM Y1210, *Cryptococcus laurentii* NCAIM Y1321, *Debaryomyces hansenii* NCAIM Y898, *Dekkera bruxellensis* NCAIM Y1007, *Geotrichum candidum* NCAIM Y274, *Kluyveromyces lactis* NCAIM Y0260, *Kluyveromyces marxianus* NCAIM Y1070, *Metschnikowia reukaufii* NCAIM Y 1120, *Pichia carsonii* NCAIM Y968, *Pichia fermentans* NCAIM Y86^{T,} *Pichia kluyverii* NCAIM Y680, *Pichia membranifaciens* NCAIM Y1044^{T}, *Rhodotorula mucilaginosa* NCAIM Y212, *Saccharomyces exiguus* NCAIM Y1033^{T}, *Torulaspora delbrueckii* NCAIM Y982, *Yarrowia lipolytica* NCAIM Y591, CBS 6124

The isolates orriginating from dairy products

Various commercial dairy products such as sweet and salt cottage cheeses, soft and hard cheeses produced by several factories were sampled repeatedly, and also different brands of the same type of products were purchased.

The strains originating from poultry

The strains (Y01481, Y01482, Y01483, Y01484, Y01485, Y01486, Y01487, Y01488, Y01489) compared to the *Yarrowia lipolytica* strains originating from dairy products were isolated from different samples of chicken breast and liver (Ismail et al., 2000).

Cheese samples for comparison of media

A roquefort-type cheese (product of Mizzo, Hungary) was purchased from a supermarket and was cut into three sub-samples and ten grams of each portion was homogenized in 90 cm^3 0,1 % pepton water. After 3 min settling, further decimal dilutions were prepared up to 10^{-6} g cm^{-3} level in duplicate from each sub-samples.

Media

Whenever possible, commercially available media were used and prepared according the manufacturer's intruction.

The following media were made: (1) Rose bengal chloramphenicol agar (RBC; Merck), (2) Dichloran rose bengal chloramphenicol agar (DRBC; Merck), (3) dichloran 18 % glycerol agar (DG18) prepared from base (Merck) with the addition of chloramphenicol (100 mgl^{-1}) selective supplement (Sigma-Aldrich). Three kind of malt extract agar were made from malt extract broth (Merck)., such as (4) malt extract salt agar (MES) supplemented with 4% NaCl and oxytetracycline (100 mg l^{-1}; Fluka), (5) malt extract biphenyl agar (MEP), supplemented with 0,05% (w/v) biphenyl (Fluka), (6) malt extract ox-bile agar (MEOX) supplemented with 0,2% (w/v) ox-bile (Fluka). (7) Oxytetracycline gentamycin glucose yeast extract agar (OGGY) was made from glucose yeast agar base (OGY, Merck) to which filter sterilized oxytetracycline (100 mg l^{-1}, Fluka) and gentamycin sulfate (50 mgl^{-1}; Fluka) were added. (8) Molibdate agar (MOL) was prepared as described by Maclaren and Armen (1958). (9) Molybdate propionate agar (MOPR) was made from MOL with the addition of 10% (w/v) calcium propionate (Fluka) solution. (10) Yeast extract glucose chloramphenicol agar with oligomycin (YGCO) was made from a commercial YGC base (Merck), whose poured plates were surface supplemented with 0,1 cm^3 filter sterilized oligomycin solution (100 mgl^{-1}, Fluka). (11) Yeast extract eugenol agar (YEE) was prepared from a commercial yeast extract agar (Merck) supplemented with eugenol (Merck) to give a final concentration of 200 μg cm^{-3}.

Counting

From each medium six plates were prepared (three sub-samples) from each of the three highest solution (10^{-4}, 10^{-5}, 10^{-6}) by speading 0,1 cm^3 aliquots of serially diluted samples ont he surface of plates. After incubation for 5 days at 25 ˚C plates were counted. Colonies were differentiated ont he basis of morphology and counts of yeast, mould and bacterial colonies recorded.

Statistical evaluation

Data were statistically analyzed using a Statgraphics program (Version 5.1; Statistical Graphics Corporation) for two-factorial analysis of variance. Significant differences in mean values of total counts between sub-samples and between media were expressed at $P<0,05$ level.

Isolation of yeasts

Dichloran rose bengal chloramphenicol agar (DRBC) was used for the selective isolation of yeasts from dairy products.

Simplified identifiction method

The recent modification of the simplified identification method (SIM) (Deák and Beuchat, 1996) was used for taxonomic assignment of isolates. Briefly, the testing regime includes, in addition to morphological investigations, analyses for urea hydrolysis, growth in the prescence of cycloheximide, fermentation of glucose and assimilation of nitrate, erythritol, cellobiose and mannitol. Additional tests may be used to discriminate the 120 most-frequent food-borne yeast species included the SIM database matrix.

DNA extraction for PFGE

Yeasts were growth in YEPD broth at 27 ˚C overnight. Cells were centrifuged (12000 rpm, 2 min) and washed in dest. water. After that the cells were pretreated with buffer (pH=8) containing of 10 mM Tris-HCl, 5 mM EDTA and 5 mM DTT for 10 minutes. After centrifugation (12000 rpm, 2 min) the cells were treated with 10 ml solution of Lysing enzyme (0,2 gr/ml) (Sigma) and 1 M sorbitol at 37 ˚C for 60 min. The protoplast were separated by centrifugation (2000 rpm 10 min) and suspended in 300-300 µl buffer (pH=8) consists of 1 M sorbitol and 250 mM EDTA. 55 mg LMA agarose (Sigma) was melted in 4,1 ml in same buffer, and plugs were made by mixing 400 - 400 µl of this solution and protoplast solutions. The plugs were treated with S3 solution contining of proteináz K (1 mg/ml) at 50 ˚C for two days. After this the plugs were washed twice in buffer (pH=9) consists of 50 mM EDTA, and stored in buffer (pH=9) consists of 0,5 M EDTA.

DNA extraction for PCR

DNA isolation was performed by the modified method of Hoffman and Winston (1987).

Pulse Field Gel Electrophoresis(PFGE, kariotyping)

A CHEF-DR II apparatus (BioRad) was used for kariotyping. *G. candidum* chromosomal DNA prepared in agarose plugs were separated in 0,9% agarose gel with 0,5 TBE buffer at 44 V, 5000 s pulse time for 75 h followed with 47 V, 3000-s pulse time for 80 h and 49 V, 2100 s for 75 h. After electrophoresis bands were visualized with ethidium-bromid and photographed (GelDoc 1000, BioRad).

Amplification of 18S rDNA with the neighbouring ITS1 region

For amplification NS1 (5'GTAGGTAGTCATATGCTTGTCTC 3') and ITS2 (5'GCTGCGTTCTTCATCGATC3') primer pair was used as described by Dlauchy et al. (1999). The PCR program consisted of the following steps: a longer initial denaturation at 95 ˚C for 3 min, 35 cycles of denaturation at 95 ˚C for 40 s, annealing at 58 ˚C for 40 s and extension at 72 ˚C for 2 min and a final extension at 72 ˚C for 3 min. The amplification reaction was performed with a thermocycler (Hybaid).

Restriction analysis

Hae III and *Msp I* restriction endonucleases were used separately to digest the amplification products of PCR.

RAPD-PCR and microsatellite-PCR

Amplification was performed using OPE 16 random primer (5'GGTGACTGTG3') and M13 microsatellite primer (5'GAGGGTGGCGGTTCT3') (Andrighetto et al., 2000) and the following amplification condition: an initial denaturation at 94 ˚C for 4 min followed by 35 cycles consisting of 30 s at 94 ˚C, 45 s at 36 ˚C, 45 s at 72 ˚C and a final extension of 7 min at 72 ˚C.

Gel electrophoresis

Both the restriction fragments of ribotyping and the amplicons of RAPD-PCR and microsatellite-PCR were separated by electrophoresis on 1,2 % (w/v) agarose gel using horizontal electrophoresis system (Hybaid). After stainig gels were digitalized using GelDoc 2000 system (BioRad). Dendograms were made based on patterns using Molecular Analysis program, UPGMA method (BioRad).

Methods used for characterization of isolates originating from poultry

Most of the morphological and physiological characteristics of the strains were determined by using conventional methods, as described by Yarrow (1998). The utilization of carbon sources was examined at 25 ˚C on a rotary shaker (30 rpm) for three week. The utilization of nitrogen sources was examined by using the conventional auxanographic technique for 7 days. The urease activity was tested on urea R broth (Difco) and the type strain of *Y. lipolytica* was included as control. The lipolytic activity was tested by the method described by Marquina et al. (1992).

Restriction enzyme analysis of the small subunit (18S) rDNA with the neighbouring ITS1 region was carried out with *Alu I*, *Hae III*, *Msp I*, *Not I* and *Sfi I* restriction endonucleases.

A part of the large subunit (26S) rDNA with the neighbouring ITS2 and 5.8S rDNA, was amplified with primers ITS3 (5'GCATCGATGAAGAACGCAGC3') (White et al., 1990) and LR5 (5'ATCCTGAGGGAAACTT3') (Vilgays and Hester, 1990). The D1/D2 domain was sequenced as described by Kurtzman and Robnett (1998). The generated DNA sequences were submitted to the GenBank and aligned using the BLAST 2.2.2 database search program (Altschul et al., 1997).

3. Summary of results

Comparison of selective media for the isolation of yeasts

Considering the efficacy in restricting mold growth, the most inhibitory was MEP in that the presence of biphenyl inhibited completely the development of molds. On the other hand, MOL, MEOX and MOPR did not supress mold development satisfactorily, hence spreading and overgrowth by molds rendered the counting of yeast colonies difficult. As to the inhibition of bacteria, calcium propionate, ox-gall and eugenol proved to be inefficient. Eliskases-Lechner and Prilinger (1996) also found that sodium propionate did not inhibit growth of bacteria, although its inhibitory effect was observed by Bowen and Beech (1967). Contrary to previous reports (Kim et al., 1995; Moleyar and Narashimham, 1992; Vazquez et al., 2001), eugenol, the main component of clove oil, did not inhibit the growth of bacteria in the final concentration of 200 μg/ml at all, so the YEE medium was mostly covered by bacteria. Plates became

slimy for bacterial development and not countable in some cases, or counting was difficult because of the presence of mixed colonies of bacteria and yeasts. Eliskases-Lechner and Prilinger (1996) using yeast-extract-glucose-chloramphenicol agar supplemented with 100 μg/ml oligomycine detected the growth of different kind of molds, including *Penicillium roquefortii*, but the colony diameter was significantly reduced. In our study YGCO with the same concentration of oligomycine while inhibiting bacteria did not restrict enough the growth of molds. Rale et al (1984) found that molybdate agar with or without calcium propionate were very useful in isolating yeast from a variety of fruits. According to the present study, molybdate media, MOL and MOPR did not support yeast growth properly and permitted the development only of minute yeast colonies.

In agreement with previous general experience, RBC, DRBC, OGGY and also DG18 supported well the growth of yeast while were inhibitory for both bacteria and molds. Large size of yeast colonies facilitated easy counting, in addition DRBC and RBC were also discriminative in colony types (Beuchat, 1993; Deák and Beuchat, 1996).

Identification of yeasts originating from dairy products based on traditional tests

Biodiversity of yeasts in the Hungarian dairy products showed a great heterogeneity. The isolates collected from various dairy products and identified with the help of SIM method.

The most frequent isolates were *Debaryomyces hansenii, Geotrichum candidum, Yarrowia lipolytica.* These dominant yeast species were commonly observed in several types of dairy products according to previous reports. Roostita and Fleet (1996) described the diversity of yeasts in Camembert and Blue-veined cheese and of 240 isolates *Debaryomyces hansenii*, *C. lipolytica*, *C. kefyr*, *C. intermedia*, *Saccharomyces cerevisiae*, *Cryptococcus albidus* and *Kluyveromyces marxianus* were the most frequent. Tempel and Jacobsen (1998) studied the occurrence of yeasts in raw milk and

during proccessing, maturation of Danablu, a traditional Danish blue-veined, semi-soft cheese. Wide range of yeasts were found to be associated with the production of Danablu, among them *Candida famata* (teleomorph: *Debaryomyces hansenii*) was the most predominant yeast, followed by *Candida catenulata.* Prillinger and coworkers (1999) isolated 76 strains, assigned to 39 species and found that *Debaryomyces hansenii*, *Geotrichum candidum*, *Issatchenkia orientalis*, *Kluyveromyces lactis*, *K. marxianus*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica* and *Candida catenulata* were the predominant in different cheeses from Austria, Denmark, France, Germany and Italy. Andrighetto and coworkers (2000) isolated 48 strains from Italian cow, buffalo, goat, and Greek ewe cheese and assigned 42 strains to species *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *K. lactis*, *Debaryomyces hansenii*, *Yarrowia lipolytica* and *Torulaspora delbrueckii*. Considering these reports, the pesent study shows that the yeast composition of Hungarian dairy products is comparable to the yeast populations of cheeses produced in other European countries. Our data agree with the species most frequently found in several types of cheese, with the exception, that we did not find representatives of *Saccharomyces cerevisiae*, *Candida intermedia*, *Cryptococcus albidus* and *Issatchenkia orientalis*.

Comparison of Geotrichum candidum strains by kariotyping

Chromosomal DNA molecules of *Geotrichum candidum* were separated succesfully by contour-clamped electric field (CHEF) PFGE and three to six bands were obtained. The most of the strain's karyotypes showed chromosomal polymorphysm, only two strain were isogenic. Characterization of polymorphic karyotypes permitted discrimination of strains. However, the drawback of karyotyping is the long time necessary to obtain results.

Species level identification of isolates by ribotyping

The genotypic characterization of the yeasts isolates by the restriction enzyme analysis of PCR-amplified rDNA (ribotyping) resulted in species-specific patterns, in that all strains belonging to same species gave a uniform digestion patterns. The results of identification by simplified identification system (SIM) were confirmed genotypically on the basis of the data of ribotyping.

In agreement with the experience of Dlauchy and coworkers (1999), we confirmed the efficacy of restriction enzyme analysis of PCR amplified 18S rDNA with the neighbouring ITS1 region (ribotyping) for rapid identification of yeasts at species level.

Comparison of isolates at strain-level using RAPD-PCR and microsatellite-PCR

Several strains belonging to the predominant *Geotrichum candidum, Debaryomyces hansenii* and *Yarrowia lipolytica* species were further studied with randomly amplified polymorphic DNA (RAPD) and microsatellite-PCR analysis. Using OPE 16 random-, and M13 microsatellite primers, RAPD-, and microsatellite-PCR produced bands differentiating between strains within the same species.

Comparison of isolates at strain-level using RAPD-PCR

On the basis of different patterns, strains of both *Geotrichum candidum, Debaryomyces hansenii* and *Yarrowia lipolytica* could be divided into several groups. Contrary to previous reports (Prillinger et al.,1999; Andrighetto et al, 2000) we did not find RAPD-, and microsatellite-PCR analysis suitable for identification at species level but it can be used for the discrimination at strain level. Considering the patterns consisting of different bands produced by same species, RAPD analysis with OPE 16 and microsatellite-PCR with M13 primer is recommended for typing of strains isolated from dairy products. However, efforts to differentiate strains according to their origin failed, and no correlation was found between the origin of yeasts and types of dairy products using these procedures. Strains

occurred with the same patterns that have been isolated from different sources.

Comparison of Yarrowia lipolytica strains to isolates originating from poultry

Yarrowia lipolytica proved to be one of the most frequent yeast species in Hungarian dairy products. Its strains were compared using molecular methods to isolates originated from an other type of food (poultry).

When the restriction fragment patterns, obtained by *Hae III* enzyme digestion of the DNA fragment amplified from 18S rDNA with NS1 and ITS2 primers from the investigated strains originated from poultry, strains from dairy products identified as *Y. lipolytica* and *Y. lipolitica* type strain were compared, we found that the six strains isolated from poutry exhibited restriction patterns which were identical to one another, but sharply different from that of the type strain of *Y. lipolytica.*

Considering the physiological properties, the most clear-cut difference which was found between the characteristics of *Y. lipolytica* (Barnett et al. 2000, Kurtzman, 1998), all investigated strains was that, unlike *Y. lipolytica,* all investigated strains were unable to grow on N-acetyl-D-glucoseamin as a sole carbon source. Furthermore, the investigated strains did not require external vitamins for growth. Some other differences were also found, however these were incostinent. For example, three of the investigated strains assimilated trechalose, while this test gave various result in the cases of the remaining three strains.

The genetic difference between the group of the six aforementioned strains on one hand and the type strain of *Y. lipolytica* on the other hand was confirmed by comparing the sequences of the D1/D2 domain of the large subunit (26S) rDNA of the strains NCAIM Y01482, NCAIM Y01486, NCAIM Y01489 and the type strain of *Y. lipolytica* NCAIM Y00591. The result of the sequencing revealed that the strain NCAIM Y01482 exhibited 42 nucleotid differences in the 500 basepair long fragment, when

compared to the type strain of *Y. lipolytica.* There was only a single nucleotid substitution between strain NCAIM Y01482 and other two strains sequenced, and exhibited a RFLP pattern identical to that of the strain NCAIM Y1482 and different from *Y. lipolytica* type strain. The sequences of the D1/D2 region of large subunit (26S) rDNA for strains Y01482 and Y1486 were deposited at the GenBank database.

4. Conclusion

A number of mycological media tested in this study cannot be recommended for use to enumerate yeasts from dairy products. Among these are MES, MEOX, MOL, MOPR, YEE, YGCO which failed to inhibit growth of bacteria and / or molds, or did not support yeast growth appropriately. DG18, OGGY, MEP, RBC and DRBC media proved to be the most efficient for the isolation of yeasts. For convenience of use, ease of preparation and counting, commercially available media such as DRBC and RBC appears to be a proper choice for the enumeration and isolation of yeasts from dairy products.

The yeast composition of Hungarian dairy products is comparable to the yeast population of dairy products produced in other European contries. The species most frequently found were *G. candidum*, *D. hansenii* and *Y. lipolytica.* In addition 24 different species were isolated in one to three cases and identified using simplified identification system (SIM; Deák, 1995).

Identification of the isolates using SIM was atchieved more rapidly than with traditional methods. This method can be convenient for the relatively rapid identification of yeasts originating from dairy products.

Strains of *Geotrichum candidum*, one of the species most frenqvently occuring in dairy products, were compared first by karyotyping. A certain degree of polymorphism of strains was observed only when an electrophoresis program lasting for 230 hours was used, because of the weak mobility of huge chromosomes. For this reason karyotyping cannot be recommended as an easy rapid

method for comparison of yeasts strains with large-sized chromosomes like those of *Geotrichum candidum*.

In agreement with previous reports, restriction analysis of PCR-amplified 18S rDNA with the neighbouring ITS1 region has been highly efficient and reliable for species identification. RAPD-, and microsatellite-PCR analysis discriminated strains at intraspecies level in majority of cases.

Nine strains of an unknown yeasts species, phenotypically resembling *Y. lipolytica* and isolated from chicken breast and liver were compared to Y. lipolytica strains fom dairy products and type strain, and the investigation of their small (18S) and large (26S) subunit rDNA revealed a robust genetic difference between six strains originating from poutry and the rest. A consistent differencein the physiological properties, suitable for preparation of the two taxa, was also found. DNA sequences of this hitherto unknown yeasts were submitted to the GenBank, and the new species, represented by these six strains was described as *Candida galli* (Péter et al., 2004).

5. The significance of investigations

Until now, no detailed and thorough molecular investigations have been made on yeasts of Hungarian dairy products. It was demonstrated by the results obtained, that yeasts regulary occured in the microbiota of Hungarian dairy products. Their contribution is essential in the production of certain dairy products, resulting in economic profit but on the other hand the loss caused by spoiling of dairy products can be considerable.

However regarding the hygienic and microbiological requirements of dairy products, the yeasts count is not regulated by orders and lows, in order to prevent or postpone the spoilage caused by them, it seems to be necessary to expand the private control of the microbiological quality of products to yeasts in dairy factories.

This underlines the importance of microbiological testing and the methodological studies presented here. By the comparison of selective media, the most suitable one has been recomended for

isolation of yeasts from dairy products. Data were obtained about the composition of yeast biota in Hungarian dairy products. The traditional and molecular methods were used and recomended for identification of yeasts isolated from dairy products. They can be differentiated on both species and strain level. All these results can be useful not only for the basic research, but also for the quality control in dairy factories, and for the assesment of spoilage risk caused by yeasts.

6. References

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