academicJournals

Vol. 8(32), pp. 3029-3037, 6 August, 2014
DOI: 10.5897/AJMR2013.6277
Article Number: EDCD15C46553
ISSN 1996-0808
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Yeasts in traditional Moroccan goat cheese

Mouna Ouadghiri[1,2], Mohamed Amar[1,2]*, Heide-Marie Daniel[3], Jean Swings[4] and Peter Vandamme[4]

[1]Laboratoire de Microbiologie et Biologie Moléculaire (LMBM), Centre National pour la Recherche Scientifique et Technique (CNRST), Rabat, Morocco.
[2]Moroccan Coordinated Collections of Micro-organisms (CCMM)/LMBM, CNRST, Rabat, Morocco.
[3]Mycothèque de l'Université catholique de Louvain, Belgian Coordinated Collection of Microorganisms, Earth and Life Institute, Applied Microbiology, Mycology, Université catholique de Louvain, Louvain-la-Neuve, Belgium.
[4]Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium.

Received 13 August, 2013; Accepted 26 February, 2014

Nine samples of goat's cheese were collected. A total of 68 yeasts were isolated and grouped according to their M13 PCR-fingerprints. Representative isolates of each fingerprint group were identified using rRNA and/or protein-coding gene sequencing leading to the identification of 18 yeast species. The dominant species were *Kluyveromyces lactis* (19.1%), *Saccharomyces cerevisiae* (11.7%), *Yarrowia lipolytica* (10.3%), *Candida parapsilosis* (10.3%), *Kazachstania unispora* (8.0%), *Kluyveromyces marxianus* (7.4%) and *Pichia fermentans* (5.9%). The yeast diversity of Moroccan goat's cheese was established using genotypic techniques which proved to be a straightforward approach for the identification of all isolates. This work yielded a well-characterized collection of yeasts from traditional and semi-industrial Moroccan goat cheeses which will be a resource of strains with specific properties.

Key words: Yeasts, goat cheese, identification, genotypic approach.

INTRODUCTION

For centuries, humans have used goats for their milk, meat and skin. Goat's milk cheeses are produced in many Mediterranean countries such as Spain. In Morocco, goat cheese is mainly prepared in a traditional and semi-industrial way. Brands of goat cheese on sale vary from one locality to another but all supermarkets offer goat cheeses (Chriqui et al., 2006). Due to the widely appreciated organoleptic and dietary characteristics, the production of goat's milk cheese has been stimulated. Goat's milk contains more easily digestible fat with higher proportions of medium-chain fatty acids and protein with higher levels of essential amino acids than cow's milk and has a higher vitamin and mineral content (Haenlein, 2001). Cheese is a very complex microbial ecosystem in which functional interactions of different partners such as lactic acid bacteria and yeasts play a major role in the ripening process (Viljoen, 2001; Mounier et al., 2008). Selection of suitable starter strains enables the cheese maker to control or modify flavour development (Beresford et al., 2001).

The traditional Moroccan goat cheese jben is a fresh cheese consisting of a salty curd obtained by spontaneous

*Corresponding author. E-mail: amar@cnrst.ma.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

fermentation at ambient temperature which may be acelerated by addition of animal or vegetable rennet (Beresford et al., 2001). The cheese is sold locally and usually consumed within a few days, but may be preserved by salting, brining and sun-drying (Rubino et al., 2004). The traditional procedure for jben making begins with the collection of raw milk in an earthenware vessel and its spontaneous fermentation at ambient temperature until coagulation which may take up to 24-72 h depending on the temperature during the summer and winter seasons, respectively. To separate the liquid whey from the curd, the curdled milk is drained in a cloth bag for two to three days or up to 10 days depending on the desired cheese consistency. The cheese can then be partitioned, salted and left to drain and ripen further. To speed up this process and to improve safety and shelf life of the product in urban settings for household use or small scale comercial manufacturing, changes to the process are made that cause differences in sensory qualities of the product. Such a change is for example the use of small plastic moulds for draining and ripening instead of performing this step in larger batches with the help of reusable cloth bags (Benkerroum and Tamime, 2004).

To help safeguarding the traditional qualities of Moroccan goat cheese, the microbial consortia of remaining traditional cheese-making need to be studied. Goat cheeses harbour bacteria and fungi in complex microbial ecosystems (Bonetta et al., 2008). The role of yeasts is not simple to define. They may play a beneficial or detrimental role by influencing the sensory characteristics through the synthesis of aromatic compounds, desired or excessive gas production, colour and texture changes, as well as surface growth and may play a role as inhibitors of undesired bacteria(Viljoen, 2001; Fröhlich-Wyder, 2003).Similar to other dairy products, a selection of yeasts is expected to be present in goat's cheeses based on shared characteristics such as fermentation or assimilation of lactose, a high proteolytic and lipolytic activity, utilisation of lactic acid, tolerance of low pH (Fleet, 1990; Jakobsen and Narvhus, 1996). The yeast species detected in raw goat milk: *Candida* spp., *Cryptococcus* spp., *Debaryomyces hansenii*, *Geotrichum candidum*, *Kluyveromyces marxianus*, *K. lactis*, *Pichia fermentans*, *Rhodotorula* spp., and *Trichosporon beigelii* could very well form the origin of some of the yeast diversity in goat milk cheeses (Callon et al., 2007; Corbo et al., 2001). Analysis of Italian soft goat's cheeses showed the presence of *Geotrichum* spp. and *K. lactis* in almost all samples, while species such as *Candida* spp., *Clavispora lusitaniae*, *Kazachstania exiguus*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* were detected less frequently in traditionally produced cheeses (Foschino et al., 2006; Bonetta et al., 2008).

While the occurrence of bacteria in goat's cheeses has been widely investigated (Requena et al., 1992; Nikolic et al., 2008; Serhan et al., 2009), the information on the yeasts in these cheeses is limited. The objective of this work is to isolate and identify, using phenotypic and genotypic techniques, yeasts occurring in traditional and semi industrial goat's cheese produced in Morocco.

MATERIALS AND METHODS

Isolation of cultures

Nine goat's cheeses samples produced traditionally (3 samples) or incooperatives (6 samples) were collected in March 2007 (Table 1). The samples were immediately cooled, brought to the laboratory and analysed upon arrival. One gram of cheese was homogenized with 9 ml of sterile physiological water (0.9% NaCl) to obtain a 1:10 dilution. Serial dilutions up to 10^{-7}were made. One hundred microliter of each dilution were surface plated on Sabouraud Agar (HiMedia, Mumbai, India) supplemented with chloramphenicol (30 µg/ml) and on Dextrose Yeast extract Peptone Agar (HiMedia, Mumbai, India) supplemented with chloramphenicol (30 µg/ml). The plates were incubated under aerobic conditions for 48 to 72 h at 30°C. The Colony Forming Unit (CFU) for both media ranged from 5.15×10^4 to 1.00×10^8. A total of 68 colonies were randomly picked from plates with 30-300 colonies. Pure cultures of yeasts were stored at -80 °C in Sabouraud broth or Dextrose Yeast extract Peptone broth with 20% (v/v) glycerol (Akabandaa et al., 2013). All strains were deposited in the CCMM/Yeast Collection under the numbers L75 to L142. A representative selection of yeast isolates has been preserved in the BCCM/MUCL collection under the numbers MUCL 52254 to MUCL 52269 and MUCL 52756 to MUCL 52761.

Physiological characterization and identification

All yeast isolates were studied using API ID 32C (BioMérieux, Marcy-L'Etoile, France) following the instructions given by the manufacturer. The software API Web (BioMérieux) was used for identification.

M 13 PCR-fingerprinting

Extraction of high-molecular weight DNA was performed by a combination of the QiagenDNeasy protocol and the Invisorb Spin Plant Mini Kit (Invitek, Germany) with modifications as described by Fidalgo-Jiménez et al. (2008). PCR fingerprinting was performed using the minisatellite specific oligonucleotide derived from the core sequence of the bacteriophage M13 with the sequence 5'-GAG GGT GGC GGT TCT-3'. PCR amplifications were performed as described by Fidalgo-Jiménez et al. (2008). The PCR fingerprint profiles were analysed using the BioNumerics software 6.1 (Applied Maths, Sint Martens Latem, Belgium). A Pearson product-moment correlation based dendrogram was generated using the UPGMA method.

Ribosomal and protein-coding gene sequence analysis

A selection of the D1/D2 region of the large subunit (LSU)rRNA, the internal transcribed spacer (ITS)rRNA sequences 1 and 2 including the 5.8S *rRNA*gene, and partial *ACT1* and *COX2* gene sequence were generated as needed to identify each isolate to the species level as described by Daniel et al. (2009) using the primers listed in Belloch et al. (2000) and Daniel and Meyer, (2003). Only sequences that showed differences to those of taxonomic reference

Table 1. Yeast species composition of Jben (n=9) from five regions of Morocco.

Jben sample	Location	Species	Number of isolates
F1, traditional	Chefchaouen	*Kluyveromyces lactis*	5
		Yarrowia lipolytica	4
F2, semi-industrial	El Gharb	*Candida inconspicua*	1
		Clavispora lusitaniae	1
		Kluyveromyces lactis	2
		Pichia fermentans	1
		Saccharomyces cerevisiae	1
F3, semi-industrial	El Gharb	*Candida inconspicua*	1
		Clavispora lusitaniae	1
		Kluyveromyces lactis	2
		Saccharomyces cerevisiae	1
F4, semi-industrial	Boufekrane	*Kluyveromyces lactis*	1
		Kluyveromyces marxianus	1
F5, traditional	Marrakech	*Candida zeylanoides*	1
		Filobasidium uniguttulatum	1
		Pichia fermentans	1
		Saccharomyces cerevisiae	3
		Torulaspora delbrueckii	1
		Yarrowia lipolytica	1
F6, semi-industrial	Rabat	*Kazachstania unispora*	4
		Kluyveromyces lactis	1
		Kluyveromyces marxianus	2
		Naumovozyma castellanii	1
F7, semi-industrial	Rabat	*Barnettozyma californica*	1
		Kasachstania unispora	2
		Kluyveromyces lactis	2
		Kluyveromyces marxianus	2
		Saccharomyces cerevisiae	3
F8, semi-industrial	Marrakech	*Candida anglica*	2
		Candida parapsilosis	2
		Candida spp.	1
		Candida zeylanoides	1
		Kazachstania servazii	1
		Pichia fermentans	2
		Yarrowia lipolytica	2
F9, traditional	Boufekrane	*Candida parapsilosis*	4
		Candida zeylanoides	1
		Filobasidium uniguttulatum	1
		Rhodotorula mucilaginosa	3

strains were deposited in public sequence databases. This were the D1/D2 LSU of isolate L124, accession number HF545834, the *ACT1* of isolate L97 accession number HF545835 and COX2 of isolate L94 accession number HF545836.

RESULTS

Physiological characterization and identification

Preliminary identification results obtained using the API ID 32Cmicrotest system were compared with those from molecular methods in Table 2. Thirty four isolates were correctly identified by API ID32C, while 14 isolates were incorrectly identified and 20 isolates could not be identified by API ID32C.

Isolates identified as *K. lactis* and *K. marxianus*, but none of the other species were able to utilize lactose as a carbon source (Table 2). In contrast, all isolates assimilated glucose and most isolates, but not *Y. lipolytica, P. fermentans, C. zeylanoides, C. inconspicua, Filobasidium uniguttulatum,* and *C. anglica* isolates, assimilated galactose. In general, the physiological profiles of different isolates within each of the species examined were similar except for those belonging to *K. lactis* and *C. parapsilosis* (Table 2).

PCR-fingerprinting

The 68 yeast isolates were typed by M13-PCR fingerprinting. Cluster analysis of the M13-PCR fingerprints (Figure 1) resulted in 14 clusters and 7ungrouped isolates (L121, L124, L129, L116, L104, L117 and L142).

Ribosomal and protein-coding gene sequence analysis

Species identification using DNA sequences was performed for 22 isolates which were selected to represent the 14 clusters and 7 single isolates found by M13-PCR fingerprinting (Figure 1). One to three of the genetic markers D1/D2 LSU rRNA, ITS rRNA, partial *ACT1* and *COX2* genes were used to identify each representative isolate unequivocally. The D1/D2 LSU region was sequenced for all 22 isolates. In addition, the ITS region was used for *C. zeylanoides*, the *ACT1* gene for *C. parapsilosis*, *S. cerevisiae*, *K. marxianus*, *K. lactis* and the *COX2* gene for *S. cerevisiae*. The highly variable *COX2* gene sequences were used to confirm strain divergence indicated by M13 fingerprints. They differed in 21 nucleotide positions from each other. While isolate L97 showed a COX2 sequence identical to MUCL 51236 (FN394076), the sequence of isolate L94 was most similar to strain MUCL 51208 (FN394075) with seven substitutions and to the neotype strain sequences AY244992, AJ295248, AF442206 with nine substitutions. According to the currently most often detected intraspecies sequence variability of 0-3 nucleotide differences in the D1/D2 LSU rRNA region, 0-4 differences in the ITS region, and 0-11 differences in the *ACT1* gene (Daniel et al., 2009 and references therein) all investigated isolates, except strain L124, were identified to the species level as reported in Figure 1 and Table 1. Strain L124 was identified as a yet undescribed *Candida* species that is related to species in the *Pichia* clade. BLAST program (Altschul et al., 1997) searches detected two highly similar sequences (two substitutions over 555 bp of the D1/D2 LSU) labelled as *Candida* spp. and isolated from an industrial malting environment in Finland (VTT C-04532, DQ377644) (Laitila et al., 2006) and from a not specified substrate in Tibet (AS 2.3080, DQ451012) (Wu and Bai, unpublished).

Yeast species diversity

Nine jben samples led to the isolation of 16 ascomycetous and 2 basidiomycetous yeast species. Each sample yielded between two and seven species (Table 1). *Kluyveromyces lactis* was present in six samples from four regions; *S. cerevisiae* in four samples of two regions; *Y. lipolytica, C. zeylanoides, K. marxianus* and *P. fermentans*in three samples of two regions; *C. parapsilosis* and *F. uniguttulatum* in two samples of two regions; *C. inconspicua, Cl. lusitaniae,* and *K. unispora* in two samples of the same region. The remaining seven species were present in single samples. There was no clear correlation between the nature of cheese analyzed (traditional or semi-industrial) and the yeast species diversity recovered. The three most frequently obtained species in terms of occurrence in different samples and number of isolates were *K. lactis* (12 isolates from six samples, 19%), *S. cerevisiae* (eight isolates from four samples, 12%) and *Y. lipolytica* (seven isolates from 3 samples, 10%).

Concerning less frequently isolated species, some samples obtained from the same location lead to the isolation of the same species: samples F2, F3 from ElGharb: *C. inconspicua*, *Cl. lusitaniae*; F5, F8 from Marrakech: *P. fermentans*, *C. zeylanoides*; F6, F7 from Rabat: *Kaz. unispora*, *K. marxianus*. Other less frequently encountered species were observed from different sample origins: *C. parapsilosis* in F8 from Marrakech and F9 from Boufekrane; *P. fermentans* in F2 from ElGharb and F5/F8 from Marrakech; *C. zeylanoides* in F5/F8 from Marrakech and F9 from Boufekrane; *K. marxianus* in F4 from Boufekrane and F6/F7 from Rabat, *F. uniguttulatum* in F5 from Marrakech and in F9 from Boufekrane.

DISCUSSION

The present study characterised physiologically and identified genotypically 68 yeast isolates originating from nine goat's cheese samples. The comparison of physiological and genotypic techniques showed that 34 isolates were misidentified when using the API ID 32C microtest system. It was noted that even species that are regularly encountered in a clinical setting, for which the API ID32

Table 2. physiological and molecular identification of yeast isolates and carbone sources assimilated.

Identified species by molecular techniques (number of isolates, cluster numbers in Figure 1)	API ID32C identification (number of isolates)	Reference	Carbone sources assimilated by isolates of the same species
Kluyveromyces lactis **(13, VIII)**	*Kluyveromyces lactis* (8)	L85, L86, L87, L82, L84, L89, L83, L90	Lactose, saccharose, galactose L84, L85, L86: lactic acid, maltose, arabinose, N-acetylglucosamine, trehalose, mannitol, sorbose L89, L90: cellobiose, raffinose, maltose, trehalose, methyl-D-glucopyranoside, sorbitol, glycerol, palatinose, melezitose, mannitol L82, L83, L87, L88, L130, L131,L85: sorbitol, mannitol
	Kluyveromyces marxianus (5)	L130, L131, L132, L130, L88	
Saccharomyces cerevisiae **(8, II and XII)**	*Saccharomyces cerevisiae* (7)	L97, L98, L141, L99, L95, L96, L94	Galactose, saccharose, lactic acid, raffinose, maltose
	KLuyveromyces lactis (1)	L93	
Yarrowia lipolytica **(7, VI)**	*Yarrowia lipolytica* (7)	L75, L79, L78, L76, L80, L77, L81	N-Acetylglucosamine, lactic acid, glycerol, erythritol, potassium gluconate
Candida parapsilosis **(6, XIV)**	*Cryptococcus humicola* (4)	L108, L110, L107, L109	All isolates except L111: galactose, saccharose, levulinic acid, N-acetylglucosamine, arabinose, maltose, trehalose, potassium 2-ketogluconate, methyl-D-glucopyranoside, mannitol, sorbitol, xylose, glycerol, palatinose, melezitose, potassium gluconate, mannitol
	Unidentified (2)	L106, L111	
Kasachstania unispora **(6, XIII)**	Unidentified (6, L104 ungrouped)	L101, L102, L103, L105, L100, L104	Galactose
Kluyveromyces marxianus **(5, IX)**	*KLuyveromyces marxianus* (5)	L134, L135, L137, L138, L136	Galactose, saccharose, lactic acid, raffinose, lactose
Pichia fermentans **(4, VII)**	Unidentified (4)	L125, L126, L127, L128	N-Acetylglucosamine, lactic acid, xylose, sorbose, glucosamine
Rhodotorula mucilaginosa **(3, I)**	*Rhodotorula glutinis* (2)	L119, L120	Galactose, saccharose, raffinose, maltose, trehalose, mannitol, ribose, glycerol, palatinose, melezitose
	Rhodotorula mucilaginosa (1)	L118	
Candida zeylanoides **(3, XI)**	*Candida zeylanoides* (2, L116 ungrouped)	L114, L116	N-Acetylglucosamine, glucosamine, potassium 2-ketogluconate, mannitol, sorbitol, glycerol, sorbose
	Unidentified (1)	L115	
Candida inconspicua **(2, IV)**	*Candida inconspicua* (2)	L112, L113	Lactic acid, glycerol, glucose
Filobasidium uniguttulatum **(2, III)**	*Candida rugosa* (1)	L122	Sorbitol, xylose, mannitol
	Filobasidium uniguttulatum (1)	L123	

Table 2. Contd.

Candida anglica **(2, X)**	Unidentified (2)	L139, L140	Glycerol
Clavispora lusitaniae **(2, V)**	Unidentified (2)	L91, L92	Galactose, saccharose, maltose, trehalose, glucosamine N-acetylglucosamine, potassium 2-ketogluconate, sorbitol, methyl-D-glucopyranoside, xylose, glycerol, rhamnose, palatinose, melezitose, potassium gluconate, mannitol, sorbose
Torulaspora delbrueckii **(1)**	*Torulaspora delbrueckii* (1)	L117	
Naumovozyma castellii **(1)**	*Zygosaccharomyces* spp (1)	L129	
Barnettozyma californica **(1)**	Unidentified (1)	L121	
Kazachstania servazii **(1)**	Unidentified (1)	L142	
Candida spp **(1)**	Unidentified (1)	L124	

Profiles of species recovered once were not shown. All isolates assimilate glucose.

has been developed, that is, *Cl. lusitaniae* and *C. parapsilosis*, were not identified. A possible explanation may be that the strains have adapted to the isolation substrate (goat cheese) and did not show the typical assimilation profiles on which the API ID32C system is based. Progress in molecular biology has opened up possibilities for characterizing yeasts at the genomic level. The application of M13 PCR-fingerprinting coupled with the sequencing of ribosomal regions and protein-coding genes proved very efficient for the identification and classification of yeasts (Kurtzman and Robnett, 1998; Daniel et al., 2009; Vrancken et al., 2010). Two M13 fingerprint types were revealed in *S. cerevisiae* (clusters II and XII), and *C. zeylanoides* (cluster XI and one ungrouped isolate) an indication of genetic heterogeneity in these species (Figure 1). This genetic heterogeneity was confirmed for *S. cerevisiae* by divergent COX2 gene sequences (Vrancken et al., 2010). One isolate of Kaz. unispora was not grouped with isolates of *Kaz. unispora* (cluster XIII). This could be a methodical artefact since the visual inspection of the its profile reveals high similarity to those in cluster XIII.

The 68 isolates represent 12 genera and 18 species (Table 1). The large species diversity recovered from the nine samples examined suggests that the genuine diversity was undersampled. However, the recurrence of the three most frequently obtained species *K. lactis*, *S. cerevisiae*, and *Y. lipolytica* among species regularly reported from cheeses (Fröhlich-Wyder, 2003) indicates the validity of the present study to determine the most frequently occurring yeasts. Italian fresh goat cheeses showed *K. lactis* in cheeses of all sampled seven producers, *G. geotrichum* with six and *Y. lipolytica* with four producers (Foschino et al., 2006) and the dominance of *Geotrichum* spp. and *K. lactis*, along with *Y. lipolytica*, *Saccharomyces* spp., *Kazachstania exiguus* and *Candida* spp. (Bonetta et al., 2008). The obtained results are comparable to the mentioned studies with the exception that no *Geotrichum* isolates were found. However, Andrighetto et al. (2000) reported only *Y. lipolytica* and *K. lactis* from Italian goat cheeses. This study did not mention sample numbers and is difficult to judge in terms of representativeness.

The basidiomycetous yeasts are non-fermentative and can be assumed not to contribute substantially to the cheese-making process. Those ascomycetous yeast species that were detected in one or two samples or with low isolate numbers only are considered as fortuitous colonisers in this study. Given the low sample and isolate number analysed here, their contribution to unique qualities cannot be excluded.

Kluyveromyces marxianus (asexual form *C. kefyr*) is one of the most frequently isolated yeast from cheese (Fröhlich-Wyder, 2003), outnumbered only by *Debaryomyces hansenii*, which has not yet been found in goat milk cheeses. The distinction of *K. marxianus* and *K. lactis* (synonym *K. marxianus* var. *lactis*, asexual form *C. shaerica*) is difficult by physiology and by molecular methods. Therefore, the current study employed *ACT1* gene sequences to distinguish both species reliably.

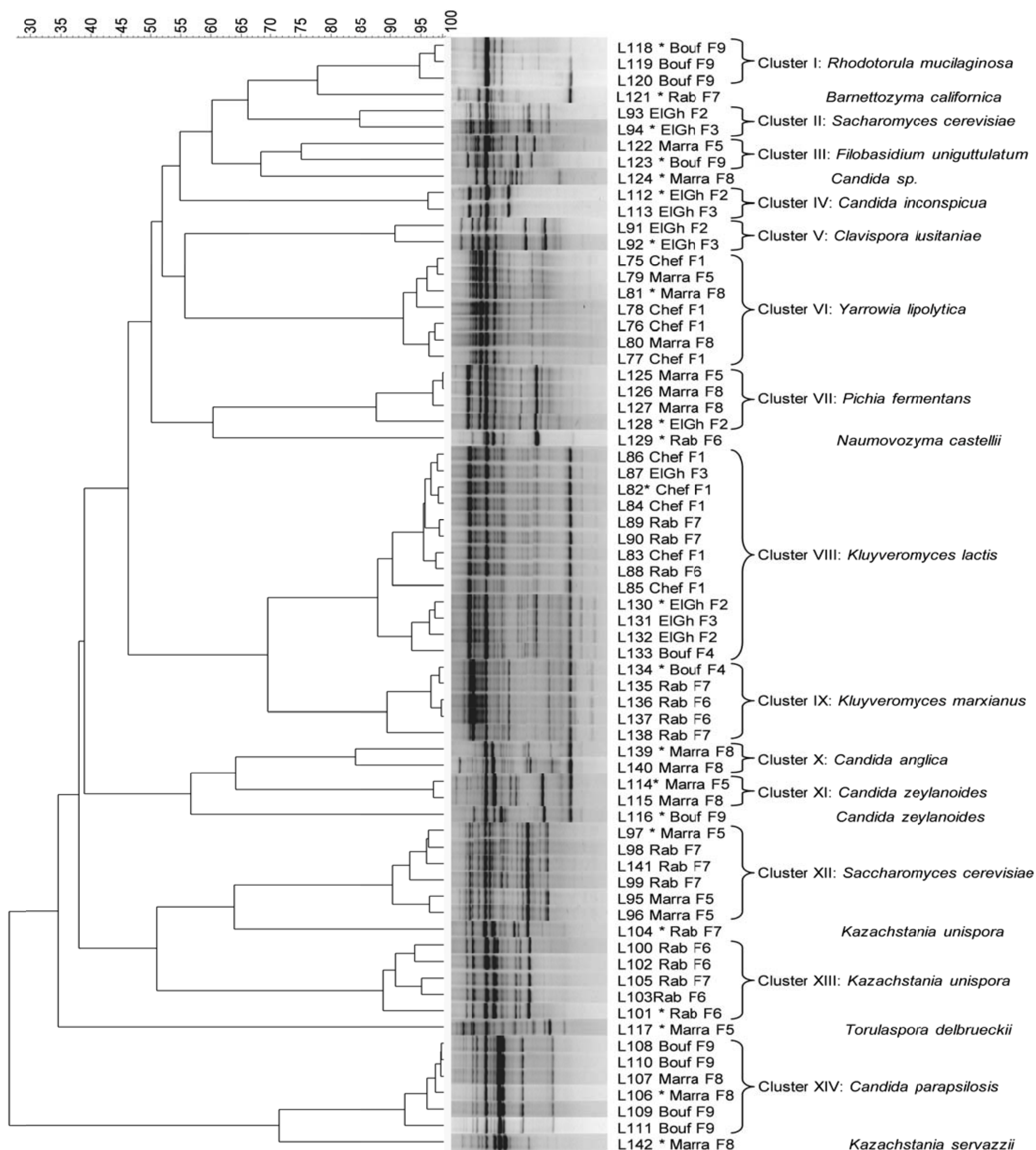

Figure 1. M13-PCR banding patterns of the yeasts isolated from Moroccan goat cheese and dendrogram based on UPGMA clustering of Pearson product-moment correlation coefficients. Isolate numbers are followed by a code for the location where the sample was obtained: Bouf = Boufekrane, Chef = Chefchaouen, ElGh = ElGharb, Marra = Marrakech, Rab = Rabat and the sample designation F1 to F9. Isolates with an asterisk were selected for sequencing analysis. Brackets designate clusters I to XIV and species identifications obtained using DNA sequence analysis are shown.

Strains of both species are able to metabolise a wide range of compounds including lactose, galactose and lactate (Kurtzman et al., 2011). Based on their similar physiology *K. marxianus* and *K. lactis* occupy very similar substrates. Notable differences are the growth of *K. lactis* on maltose, trehalose, methyl-α-glucoside and up to maximal 37°C, while *K. marxianus* is able to grow up to 45°C, assimilates inuline, has a higher growth rate, but does not grow on the before mentioned carbon sources (Kurtzman et al., 2011).

Saccharomyces cerevisiae is among the four most frequently reported yeasts from cheese (Fröhlich-Wyder, 2003). This species is frequently reported together with *K. marxianus* and *K. lactis* from pasta filata cheeses, which are marked by mild aromas (Romano et al., 2001). The generally lactose-negative *S. cerevisiae* (Kurtzman et al., 2011), proved to show strain variation in this ability, as for example a cheese isolate was shown to assimilate lactose and to degrade casein (Hansen and Jacobsen, 2001; Hansen et al., 2001). A positive growth stimulation of the main starter *Penicillium roqueforti* by *S. cerevisiae* was suggested by the same studies through a faster accumulation of aroma effective compounds and a more favourable sensory analysis of the cheeses produced with the combined starter relative to single starter conventional production. In general, the growth of *S. cerevisiae* in dairy product is assumed to be supported by utilisation of galactose.

Yarrowia lipolytica has been reported from cheese with a similar frequency as *S. cerevisiae* (Fröhlich-Wyder, 2003) and has been detected in goat milk cheeses repeatedly (Tornadijo et al., 1998; Foschino et al., 2006; Bonetta et al., 2008). The species is best known for extracellular enzyme production, in particular of lipolytic and proteolytic activities that lead to an either beneficial or spoilage role depending on the type of cheese (Suzzi et al., 2001). Considerable strain variations in enzymatic activities were ascribed to the strain diversity, and may, in view of recently evidenced species-level genetic variation, partly also be due to cryptic species existing in *Y. lipolytica* (Knutsen et al., 2007). Growth of *Y. lipolytica* in dairy products is thought to be supported in some strains by galactose and generally by lactic acid (Kurtzman et al., 2011), present in cheese through the action of lactic acid bacteria.

Typical cheese-associated yeasts are known to produce characteristic flavour-effective volatile sulphur compounds, free fatty acids, short-chain ketones and others. Comparisons of the major cheese-associated yeast species showed different amounts and compositions of species and strains (Sorensen et al., 2011; Kagkli et al., 2006). These results underline the importance to reproduce a species diversity in potential starter cultures that resembles the diversity found in typical traditional products as it may be impossible to imitate the regionally as typical perceived flavours by single-culture starters. The typical cheese yeasts diversity originated from unpasteurised goat milk or from the environment production (Boutrou and Guéguen, 2005). With the standardisation of food production and increasing hygiene requirements both sources may no longer contain the appropriate organisms and the application of wild starter cultures gains importance.

The results of the present study represent the first molecular analysis of the yeast microbiota of traditional and semi-industrial Moroccan goat cheese and revealed a considerable diversity. Because of the complex microbiota, and the interactions between different microorganisms with the cheese environment, species and strain selection for flavour improvement is not straightforward. Further evaluation of the metabolic properties of strains isolated in this study would improve the understanding of the Moroccan goat's cheese microbial ecosystem and support the selection of adequate starters for the industrial production of goat's cheese.

The present study is the first report on the molecular identification and physiological characterization of the dominant yeast species occurring in traditional and semi-industrial goat's cheeses produced in Morocco.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by the Belgian General Administration for International Cooperation (project: FRAB/2005) and the Centre National pour la Recherche Scientifique et Technique, Rabat, Morocco (Programme d'urgence 2009 - 2012). We acknowledge Mrs. El Khedri Hanane for helping us doing sampling and isolation of yeasts.

REFERENCES

Akabandaa F, Owusu-Kwartenga J,Tano-Debraha K,Gloverb RLK,Nielsenc DS, Jespersenc L (2013). Taxonomic and molecular characterization of lactic acid bacteria and yeasts in nunu, a Ghanaian fermented milk product.Food Microbiol. 34(2):277-283.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25(17):3389-3402

Andrighetto C, Psomas E, Tzanetakis N, Suzzi G, Lombardi A (2000). Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products. Lett. Appl. Microbiol. 30:5-9.

Belloch C, Querol A, Garcia MD, Barrio E (2000). Phylogeny of the genus *Kluyveromyces* inferred from the mitochondrial cytochrome-c oxidase II gene. Int. J. Syst. Evol. Microbiol. 50:405-416.

Benkerroum N, Tamime AY (2004). Technology transfer of some

traditional dairy products (lben, jben and smen) to small industrial scale. Food Microbiol. 21:399-413.

Beresford TP, Fitzsimons NA, Brennan NL, Cogan TM (2001). Recent advances in cheese microbiology. Int. Dairy J.11 (4-7):259-274.

Bonetta S, Bonetta S, Carraro E, Rantsiou K, Cocolin L (2008). Microbiological characterisation of Robiola de Roccaverano cheese using PCR-DGGE. Food Microbiol. 25:786-792.

Boutrou R, Guéguen M (2005). Interests in *Geotrichumcandidum* for cheese technology. Int. J. Food Microbiol. 102(1:1-20).

Callon C, Duthoit F, Delbès C, Ferrand M, Le Frileux Y, De Crémoux R, Montel MC (2007).Stability of microbial communities in goat milk during the lactation year: molecular approaches. Syst. Appl. Microbiol. 30:547-560.

Chriqui A, Alaoui A, Fagouri S, Choukri R (2006).Étude de marché du fromage de chèvre. DPA Chefchaouen ISCAE ANOC.

Corbo MR, Lanciotti R, Albenzio M, Sinigaglia M (2001). Occurance and characterisation of yeasts isolated from milks and dairy products of Apulia region. Int. J. Food Microbiol. 69:147-152.

Daniel HM, Meyer W (2003). Evaluation of ribosomal RNA and actin gene sequences for the identification of ascomycetous yeasts. Int. J. Food Microbiol. 86:61-78.

Daniel HM, Vrancken G, Takrama JF, Camu N, De Vos P, De Vuyst L (2009). Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS Yeast Res. 9:774-783.

Fidalgo-Jiménez A, Daniel HM, Evrard P, Decock C, Lachance MA (2008). Metschnikowiacubensis sp. nov., a yeast species isolated from flowers in Cuba. Int. J. Syst. Evol. Microbiol. 58:2955-2961.

Fleet GH (1990). Yeasts in dairy products. J. Appl. Bacteriol. 68:199-211.

Foschino R, Picozzi C, Borghi M, Cerliani MC, Cresci E (2006). Investigation on the microflora of Caprino Lombardo cheese from raw goat milk. Ital. J. Food Sci. 18:33-49.

Fröhlich-Wyder MT (2003). Yeasts in dairy products. In: Yeasts in Food Ed. T. Boekhout& V. Robert, Behr's Verlag, Hamburg, Germany.

Haenlein GFW (2001). Past, present, and future perspectives of small ruminant dairy research. J. Dairy Sci. 84:2097-2115.

Hansen TK, Jakobsen M (2001). Taxonomical and technological characteristics of *Saccharomyces* spp. associated with blue veined cheese. Int. J. Food Microbiol.69:59-68.

Hansen TK, van den Tempel T, Cantor MD, Jakobsen M (2001).*Saccharomyces cerevisiae* as a starter culture in Mycella. Int. J. Food Microbiol. 69:101-111.

Jakobsen M, Narvhus J (1996). Yeasts and their possible beneficial and negative effects on the quality of dairy products. Int. Dairy. J. 6: 755-768.

Kagkli DM, Bonnarme P, Neuvéglise C, Cogan TM, Casaregola S (2006). L-Methionine Degradation Pathway in *Kluyveromyces lactis*: Identification and Functional Analysis of the Genes Encoding L-Methionine Aminotransferase. Appl. Environ. Microbiol. 3330-3335.

Knutsen AK, Robert V, Poot GA, Epping W, Figge M, Holst-Jensen A, Skaar I, Smith MTh (2007). Polyphasic re-examination of *Yarrowialipolytica* strains and the description of three novel *Candida* species: *Candida osloensis* sp. nov., *Candida alimentaria* sp. nov. and *Candida hollandica* sp. nov. Int. J. Syst. Evol. Microbiol. 57:2426-2435.

Kurtzman CP, Fell J, Boekhout T (2011). The yeasts: a taxonomic study. Elsevier, Amsterdam.

Kurtzman CP, Robnett CJ (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73: 331-371.

Laitila A, Wilhelmson A, Kotaviita E, Olkku J, Home S, Juvonen R (2006). Yeasts in an industrial malting ecosystem. J. Ind. Microbiol. Biotechnol. 33: 953-966

Mounier J, Monnet C, Vallaeys T, Arditi R, Sarthou AS, Hélias A, Irlinger F (2008). Microbial interactions within a cheese microbial community. Appl. Environ. Microbiol. 74: 172-181.

Nikolic M, Terzic-Vidojevic A, Jovcic B, Begovic J, Golic N, Topisirovic L (2008). Characterization of lactic acid bacteria isolated from Bukuljac, a homemade goat's milk cheese. Int. J. Food Microbiol. 122(1-2): 162-170.

Requena T, De la Fuente MA, Fernandez de Palencia P, Juarez M, Pelaez C (1992).Evaluation of a specific starter for the production of semi-hard goat's milk cheese. Lait 72:437-448.

Romano P, Ricciardi A, Salzano G, Suzzi G (2001). Yeasts from water buffalo Mozarella, a traditional cheese of the Mediterran area. Int. J. Food Microbiol. 68:45-51.

Rubino R, Morand-Fehr P, Sepe L (2004). Atlas of goat products. La Biblioteca di Caseus, Potenza.

Serhan M, Cailliez-Grimal C, Borges F, Revol-Junelles AM, Hosri C, Fanni J (2009). Bacterial diversity of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. Food Microbiol.26: 645-652.

Sorensen LM, Gori K, Petersen MA, Jespersen L, Arneborg N (2011). Flavour compound production by *Yarrowialipolytica*, *Saccharomyces cerevisiae* and *Debaryomyceshansenii* in a cheese-surface model. Int. Dairy J. 21(12):970-978.

Suzzi G, Lanorte MT, Galgano F, Andrighetto C, Lombardi A, Lanciotti R, Guerzoni ME (2001). Proteolytic, lipolytic and molecular characterisation of *Yarrowialipolytica* isolated from cheese. Int. J. Food Microbiol.69:69-77.

Tornadijo ME, Fresno JM, Martin Sarmiento R, Carballo J (1998). Study of the yeasts during the ripening process of Armada cheeses from raw goat's milk. Lait 78:647-659.

Viljoen BC (2001). The interaction between yeasts and bacteria in dairy environments. Int. J. Food Microbiol. 69:37-44.

Vrancken G, De Vuyst L, Van der Meulen R, Huys G, Vandamme P, Daniel HM (2010). Yeast species composition differs between artisan bakery and spontaneous laboratory sourdoughs. FEMS Yeast Res. 10:471-481.