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Isolation and physiological characterization of indigenous yeasts from some Algerian agricultural and dairy products

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The purpose of this study was to isolate yeasts that may express original characteristics notably in terms of flavour from traditional Algerian agricultural and dairy products. A total of eighteen yeast isolates were recovered from dates, melons and gherkins, and from milk of camel, goat, sheep and cow. Molecular taxonomy based on the sequences of the D1/D2 domain of the 26S ribosomal RNA gene, grouped these isolates into nine yeast species. The identification of *Clavispora lusitaniae, Hanseniaspora uvarum, Kodamaea ohmeri, Pichia kudriavezii*, *Zygosaccharomyces baili*, *Zygosaccharomyces rouxii* and *Yarrowialipolytica* was in accordance with the physico-chemical characteristics of agricultural and dairy products analyzed. The *P. kudriavezii* species showed resistance to ethanol and osmotic stress, as well as production of alcohols that was remarkably higher than the laboratory *S. cerevisiae* strain CEN.PK. In conclusion, this study draws attention to the use of this yeast species for biotechnological application in the field of flavour production.

Key words: Yeast taxonomy, microbial physiology, stress, fermentation, flavours.

INTRODUCTION

For millenniums, traditional industrial biotechnology has used yeasts and filamentous fungi in the production of large variety of consumer goods like beer, wine and fermented foods. Nowadays, there is a shift towards modern industrial biotechnology, also termed white biotechnology, to harness energy and produce bio-based chemicals from renewable carbon sources using microorganisms (Octave and Thomas, 2009). There is also a growing interest in the biodiversity and ecology of yeasts associated with different foods either as biocontrol agents of food spoilage (Fleet, 2007) or as new vectors for producing metabolites of high added value such as flavours and aromas (Berger, 2009). Potential relevance of these studies could be to exploit these original traits expressed by these 'wild' yeasts in biotechnological applications, such as microbial production of flavour compounds (Lomascolo et al., 1999), isoprenoids derivatives for use in biofuel (Kirby and Keasling, 2008) or to transfer genes bearing specific traits into genetically tractable hosts to enhance their physiological performance (Fortman et al., 2008).

The remarkable development in molecular techniques associated with the ease of DNA sequencing allows a rapid investigation of the biodiversity and ecology of microorganisms isolated from traditional food, beverages or other natural biotopes. Current methods for yeasts

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identification are based on restriction-fragment length polymorphism of 5.8S-ITS rDNA region or sequencing of the D1 and D2 domains in the 26S rDNA gene (Kurtzman and Robnett, 1998). The purpose of this work was to explore the biodiversity of yeasts in traditional Algerian food and dairy products. We reported on the isolation of 9 different yeast species isolated from these traditional Algerian agricultural products, and investigated five out of these nine isolates for their growth performance on various sugars, resistance to high ethanol and osmotic pressure as well as for their capacity to produce specific aroma and flavours in comparison to the well-studied laboratory yeast *Saccharomyces cerevisiae* CEN.PK122-2N (van Dijken et al., 2000).

MATERIALS AND METHODS

Biological sampling and isolation of yeasts

The following products were collected from local markets in different Algerian cities: three varieties of dates (Deglet-Nour from Ghardaïa; Boufakous-gharess from Bechar and Hamira from Ourgla), milk of cow, goat, sheep and camel were from Ghardaïa; melon and gherkins from a local market at Oran and honey from Mostaganem. Yeasts were isolated from dates, gherkins and melons after grounding them in a sterile mortar, collecting one gram of the paste and mixing it with 9 ml of 2% (w:v) sodium citrate. The resulting solution was serially diluted in sterile water and 0.1 ml was spread on a solid OGA containing 2% (w/v) glucose, 0.5% (w/v) yeast extract, 2% (w/v) agar supplemented with 0.5 mg/ml chloramphenicol, 0.1 mg/ml oxytetracycline and 3 mg/ml thiabendazole. The plates were incubated at 30°C for three days. Isolation of yeast species from milk of camel, cow, goat and sheep was obtained after its coagulation by lactic acid fermentation. Following this step, the samples were checked for the apparition of yeasts under the microscope and using gentian violet crystal. Yeast colonies showing different size, shape and/or color were picked up, examined under the microscope, and finally isolated by an enrichment technique in OGA plates as above. Isolated colonies were streaked and stored at 4°C on Sabouraud agar slants (2% (w/v) glucose, 1% (w/v) bactopeptone, 1.5% (w/v) agar and 0.5 mg/ml chloramphenicol. After molecular identification, the yeast species were stored at -80°C in YEPD medium containing 25% (v/v) glycerol.

Identification of yeast isolates

The molecular identification of yeast isolates was carried out by the "Centre International de Ressources Microbienne" (CIRM; http://www7.inra.fr/cirmlevures/page.php?lang=fr&page=home) in Thiverval Grignon, Paris. The molecular taxonomy employed the procedure developed by Kurtzmann and Robnett (1997, 1998). The method is based on PCR amplification of the D1 and D2 domain of the large subunit (26S) rRNA gene followed by sequencing and comparison of the nucleic sequences using a BLAST search (Altschul et al., 1997) with the GenBank database of NCBI.

Culture media and growth conditions

Unless otherwise stated, yeast cultures were carried in 1 L Erlenmeyer flasks containing 0.30 L of either glucose rich medium (YEPD) (20 g glucose, 10 g yeast extract, 10 g Bactopeptone per liter), glucose-synthetic medium (SD) (20 g glucose, 1.7 g Yeast nitrogen base without amino acids and ammonium; 5 g ammonium sulphate per liter) set at pH 5.0 with succinic acid (1.35% w/v) and NaOH (0.65% w/v); or glucose-synthetic mineral medium (SMM) made according to Verduyn et al. (1992). The flasks were agitated at 30°C on a rotary shaker set at 200 rpm. For solid media, agar was added to YEPD or SD medium to reach 2% (w/v). Anaerobic growth was carried out in 50 ml-capped vials containing 25 ml of SD medium complemented with 0.05% (v/v) Tween 80; 0.01% (w/v) ergosterol (from a 1000x stock solution made in isopropanol and in the presence of 1 g/l Resazurin sodium salt as oxygen indicator).

Phenotypic analysis

The growth capacity on various carbon sources was carried out using the commercial available API:ID 32C gallery (purchased from BioMérieux SA, France). This micro-method allowed a rapid survey of the carbon assimilation and hence on the metabolic ability of the yeast isolates. Phenotypic assays for various stresses were performed on YEPD or SD agar plates. Prior to the assays, yeast cells were cultivated in YEPD at 30°C for 24 h. After reading the optical density (OD) at 600 nm, 1 ml of the culture was collected and concentrated to 8.0 OD_{600}. Then, 5 µl from a serial dilution made in sterile water was spotted on the agar plates containing NaCl, sorbitol, ethanol or 2-phenylethanol at concentration indicated in the figures. Unless otherwise stated, the plates were incubated at 30°C, and the growth was scored after two days.

Analytical methods

Glucose, glycerol, ethanol and acetate were determined in the cell-free supernatant with commercial biochemical kits or by high performance liquid chromatography. In the latter case, the supernatant was filtered through 0.22 µm- pore-size nylon filters prior to loading on a HPX-87H Aminex ion exclusion column. The column was eluted at 48°C with 5 mM H2S04 at a flow rate of 0.5 ml/min and the concentration of the compounds was determined using a Waters model 410 refractive index detector. For quantification of higher alcohols, these volatile compounds were first extracted by mixing 1 ml of the culture medium with 500 µl of diethylether in the presence of octanol at 0.1% (v/v) as internal standard. After vigorous vortexing and centrifugation at 1000 g for 5 min, 2 µl of the organic phase was injected on an Agilent 6890N gas chromatograph system equipped with a flame ionisation detector (FID). A HP5 capillary GC column (Agilent Technologies) with dimensions of 30 m length x 0.32 mm internal diameter with a 0.25 µm film thickness was used for separation. The injector temperature was set a 250°C with a split ratio at 1:10. carrier gas was helium. The oven temperature program was as follows: 50°C for 1 min; rise to 250°C at 30°C per min and set at 250°C for 5 min. Detection with FID was performed at 270°C with 30 ml/min hydrogen and 300 ml/min air flow rates. Data acquisition and processing were performed using the Chromeleon Chromatography Management System (Thermo Scientific Dionex). For each compounds analysed, a calibration curve was constructed using known amount of the pure standards (purchased from Aldrich-Sigma).

RESULTS AND DISCUSSION

Diversity of yeasts in Algerian food and dairy products

About 18 yeast-like colonies were recovered from food

Table 1. Identification of yeast isolates from Algerian fruits and dairy products.

Isolates code	Food or dairy origin	Species attributed	Identity match with NCBI
AH1 (S1)	Dates (Ghardaia)	*Clavispora lusitaniae*	500/500 (100%)
AJ3 (S2)	Dates (Bechar)	*Hanseniaspora uvarum*	525/527 (99%)
BFK (S3)	Dates (Ourgla)	*Kodamaea ohmeri*	487/487 (100%)
MJ2 (S5)	Melon (Oran)	*Candida parapsilosis*	535/535 (100%)
LVG/PC (S6)	Cow melk (Ghardaïa)	*Yarrowia lipolytica*	525/525 (100%)
LVB/PR (S7)	Cow melk (Oran)	*Yarrowia lipolytica*	525/525 (100%)
LBN/CR (S10)	sheep milk (Ghardaia)	*Yarrowia lipolytica*	525/525 (100%)
LCT/CV (S11)	Goat milk (Ghardaia)	*Yarrowia lipolytica*	525/525 (100%)
LCS/ CS (S14)	Camel milk (Ghardaïa)	*Pichia kudriavezii (formely Issatchenkia orientalis)*	526/526 (100%)
LCW/GC (S15)	Camel milk (Bechar)	*Trichosporon asahii*	563/563 (100%)
C/PC2 (S17)	Gherkins (oran)	*Zygosaccharomyces bailii*	545/545 (100%)
MM2 (S18)	honey (Mostaganem)	*Zygosaccharomyces rouxii*	543/543 (100%)

The molecular taxonomy of the yeast isolates was based on the sequencing of the domain D1/D2 of the large subunit rRNA gene 26S and comparison of the sequences with NCBI database, as described in Materials and Methods.

and dairy products collected in local markets of different Algerian cities, grouped into nine yeast species according to genomic analysis of the PCR- amplicon sequence from domain D1/D2 of the 26S rDNA gene (Kurtzman and Robnett, 1998). *Pichia kudriavezvii* (synonymus of *Issatchenkia orientalis)* and *Trichosporon asahii*were isolated from camel milk, *Clavispora lusitaniae*, *Hanseniaspora uvarum* and *Kodamaera ohmeri*from dates, *Zygosaccharomyces bailii* from gherkins, *Candida parapsilosis* from melons, *Zygosaccharomyces rouxii* from honey, and *Yarrowia lipotytica* species was the prominent yeast in cow, goat and sheep milk (Table 1). Since *T. asahii* is considered as an opportunistic pathogen whose main habitat is the skin of humans and animals (Ebright et al., 2001), probably this yeast arose during the isolation procedure. In contrast, the isolation of *Y. lipolytica* in milk of camel, cows and sheep was not unexpected due to the high content in these dairy products of lipids, a preferred carbon source for this yeast species. Also, the presence of the sugar tolerant *Z. rouxii* in honey was expected as it is very rich in sugars and so highly osmotic.

The presence of the other yeast species, notably *P. kudriavezii* in camel milk and *C. lusitaniae* in dates were less expected. *P. kudriavezii* species is one of the indigenous yeast present in wine (Clemente-Jimenez et al., 2004) and is characterized by high acidic and ethanol tolerance (Okuma et al., 1986). Thus, the presence of this species might be explained by the fact that the isolation procedure came after lactic fermentation of the camel milk. *C. lusitaniae* is a yeast species that can be found in a broad array of plants and animals substrates, as well as in industrial wastes (Lachance et al., 2003), but has been also isolated in clinical specimens (Gargeya et al., 1990). The isolation of *H. uvarum* from dates maybe in accordance with the fact that this yeast species is commonly found on grapes skin that are very sweet fruit, very rich in glucose and fructose. On the other hand, the presence of *K. ohmeri* in dates in less obvious since this yeast-like fungus is known to be vectored by insects and bees during their visit on ephemeral flowers (Lachance et al., 2001).

There is also recent report on the emergence of this yeast species as an important etiologic agent of fungemia in immuno-compromised patients (Al Sweih et al., 2011). This leaves the finding of this yeast species from dates questionable as to whether it is a truly indigenous yeast species present in dates or it has been isolated by accident during the isolation procedure. This question remains to be answered.

Growth behaviour of representative yeast isolates from Algerian agricultural and dairy products

As a result of good genetic, genomic and physiological knowledge on *Y. lipolytica* (Barth and Gaillardin, 1997), *Z. bailii* and *Z.rouxii* (Merico et al., 2003; Martorell et al., 2007), we addressed some characterization of the 5 remaining yeast isolates, namely *C. lusitaniae*, *H. uvarum*, *K. ohmeri*, *P. krudiavezii* and *T. asahii*. Growth studies were carried out on API: ID 32C galleries (Table 2). As expected, all yeast species grew very actively on glucose and galactose as carbon source. *C. lusitaniae* and *T. asahii* were also able to grow on C5-sugars such as arabinose, xylose and ribose, while the *H. uvarum* had the shortest sugars spectrum for growth, as it did not grow on mannose, sucrose, maltose, trehalose or raffinose (Table 1). This qualitative analysis showed that the carbon sources spectrum of the *P. kudriavezii* species for growth was similar to that of the laboratory *S. cerevisiae* CEN.PK122-2N. Also, the *P. kudriavezii* yeast

Table 2. Growth tests of yeast species on several carbon source using API-galleries.

ID name	CEN.PK122-2N	*Clavispora lusitaniae*	*Hanseniaspor auvarum*	*Kodamaea ohmeri*	*Pichia kudriavezii*	*Trichosporo nasahii*	Substrate (from ID name)
GAL	++	+++	++	+++	++	+++	D-Galactose
ACT	+	-	++	-	-	+++	Actidione
SAC	++	+++	-	+++	+++	+++	D-Saccharose
NAG	+	+++	-	+++	+++	+++	N-Acetyl-Glucosamine
LAT	++	+++	-	+	+++	+++	Lactique acid
ARA	+	++	-	-	-	++	L-Arabinose
CEL	+	+++	++	+++	-	+++	D-Cellobiose
RAF	++	++	+	+++	-	-	D-Raffinose
MAL	++	+++	-	+++	++	+++	D-Maltose
TRE	++	+++	-	+++	-	+++	D-Trehalose
2KG	+	++	++	+++	-	+++	Potassium 2-ketoglutamate
MDG	+++	+++	-	+++	-	+++	Methyl-αD-Glucopyranoside
MAN	+	+++	-	+++	+	+	D-Mannitol
LAC	+	-	-	-	-	+++	D-Lactose
INO	-	-	-	-	-	-	Inositol
O	0	0	0	0	0	0	No substrat
SOR	-	++	-	+++	-	-	D-Sorbitol
XYL	-	+++	-	-	-	+++	D-Xylose
RIB	-	++	++	+	-	+++	D-Ribose
GLY	-	+++	-	+++	++	-	Glycerol
RHA	-	+++	-	+	-	+	L-RhamnosE
PLE	+++	+++	-	+++	++	+++	Palatinose
ERY	-	-	-	-	-	++	Erythritol
MEL	-	-	-	-	-	-	D-Melibiose
GRT	-	++	-	-	-	+++	sodium GlucURONATE
MLZ	+++	+++	-	+	++	+	D-MELEZITOSe
GNT	-	++	++	-	++	+++	Potassium Gluconate
LVT	-	++	-	+	-	-	Levulinicacid
GLU	+++	+++	++	+++	+++	+++	D-glucose
SBE	-	+++	-	++	-	+	L-SorBOSE
GLN	-	++	-	++	-	++	Glucosamine
ESC	-	++	++	++	-	-	Esculine (fecitrate)

The growth was scored after 24 h at 32°C. The scoring legend is: -, no growth; +, weak biomass; ++, good biomass; +++ , high biomass.

isolated in this work was likely genetically different from the one isolated from cane syrup by Gallardo et al. (2011) which was unable to grow on sucrose and from the DMKU-3ET15 strain isolated from pork sausage fermentation which could not grow on galactose, maltose, sucrose or melizitose (Yuangsaard et al., 2013).

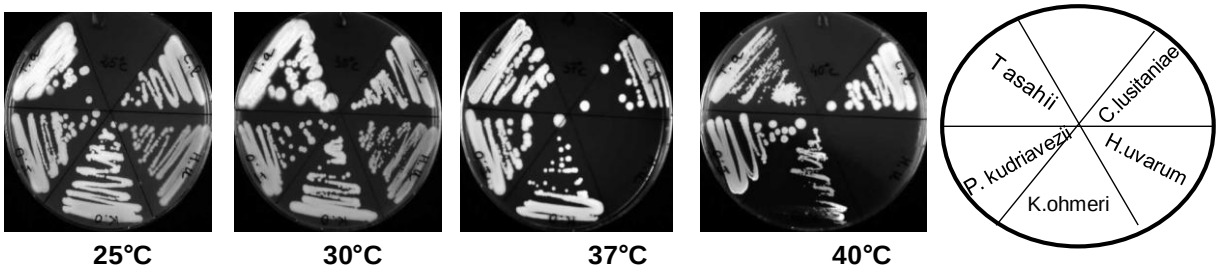
It was also found that all yeast species except *T. asahii* could grow under anaerobiosis (data not shown). Based on these qualitative growth assays, we have determined the maximal growth rate of these five yeast species cultivated on a glucose-rich (YEPD) medium (Table 1). With the exception of *T. asahii*, they grew all at the rate of about 10 to 25% faster than *S. cerevisiae* CEN.PK122-2N. In addition, they fermented glucose into ethanol, with *P. kudriavezii* and C. *lusitaniae* showing products fermentation yields very close to that of *S. cerevisiae* CEN.PK122-2N, whereas *T. asahii* showed an apparent respiratory metabolism of glucose (Table 3).

Similar results were found using a glucose synthetic mineral medium made according to Verduyn et al. (1992),

Table 3. Maximal growth rate (μ), and main byproducts formation of the various yeast species cultivated in a glucose-rich medium (YEPD).

Strain	μ (h^{-1})	Ethanol (g/l)	Yield (ethanol/glucose) (g/g)	Glycerol (g/l)	Acetate (g/l)
S. cerevisiae CEN.PK122-2N	0.45	8.5	0.42	0.55	0.22
C.lusitaniae	0.58	6.3	0.32	0.4	0.11
H. uvarum	0.50	5.3	0.27	0.65	0.24
K.ohmeri	0.56	5.9	0.30	0.4	0.07
P.kudriavezii	0.68	6.8	0.40	2.2	0.05
T. asahii	0.30	0.55	0.03	0.54	0.01

Growth was carried at 30°C. Samples were taken at the time of complete exhaustion of glucose (initial amount was 20 g/l) for analysis of the byproducts by HPLC as described in Materials and Methods. The results are the mean of 2 independent experiments.



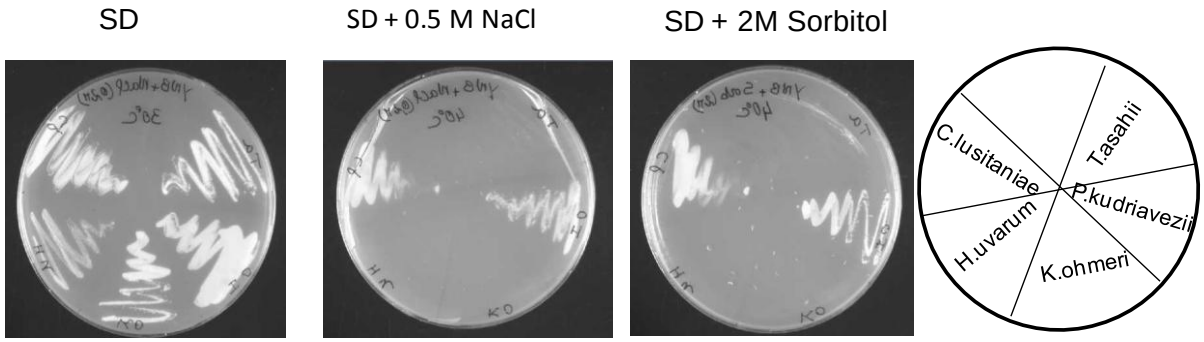


Figure 1. (A) Growth of yeast isolates on YEPD agar plates at different temperature; (B) Effect of NaCl 0.5 M or sorbitol 2 M on the growth of yeast isolates on SD agar plates at 40°C. Growth was scored after two days

except that the growth of *H. uvarum* on this medium required the supplementation of vitamins B2 (riboflavin), B3 (niacin) and B9 (folic acid) (data not shown).

Analysis of tolerance to stressing conditions

Growth dependencies on temperature and pH were determined for the 5 yeast species retained in this study. Except *H. uvarum* that was unable to sustain growth above 30°C, the other four yeast species grew pretty well up to 40°C (Figure 1A). We noticed that the growth of *P. kudriavezii* was the least affected at high temperature, in agreement with the thermotolerant properties of this yeast species reported in previous studies (Gallardo et al., 2011; Kwon et al., 2011; Yuangsaard et al., 2013). In addition, this yeast species displayed the widest range of pH for growth (pH 2.5 to 7), whereas *T. asahii* and *K ohmeri* were unable to grow at pH equal or lower than 2.5

(data not shown). These yeast species were also tested for salt and osmotolerance. It was found that only *C. lusitaniae* and *P. kudriavezii* were capable of growing on glucose medium supplemented with 0.5 M NaCl or 2 M sorbitol even at 40°C (Figure 1B). *P. kudriavezii* was also found to be the most resistant yeast species to ethanol toxicity since it could still grow in the presence of 18% ethanol, whereas the growth of *S cerevisiae* CEN.PK122-2N strain started to be impaired at 12% ethanol, and that of the other yeast species was inhibited at concentration equal or above 8% ethanol (Figure 2). In line with the high resistance of *P. kudriavezii* to ethanol, we found that this yeast species was about two times more resistant than *S. cerevisiae* to 2-phenylethanol (2-PE), a relevant rose-like flavour produced by the Erlich pathway (Hazelwood et al., 2008) (Figure 3). Like ethanol, 2-PE can diffuse freely in and out of the cell and can disturb cell membrane integrity (Silver and Wendt, 1967; Lloyd et al., 1993; Hua and Xu, 2011). Although, the molecular mechanism underlying ethanol resistance is not yet fully understood (Stanley et al., 2010), a role of membrane composition and fluidity has been proposed to be crucial for this resistance (Ding et al., 2009). Thus, investigation of the membrane fluidity and determination of the phospholipids membrane composition in this yeast species might provide some clues to a better understanding of the resistance of *P. kudriavezii* to ethanol and 2-PE and to eventually exploit this knowledge to increase the tolerance of the yeast *S. cerevisiae* to these alcoholic molecules.

Analysis of the production of higher alcohols

We were also interested in determining the capacities of the yeast isolates for the production of biotechnological relevant products, such higher alcohols and 2-phenylethanol (Berger, 2009). The yeast species were cultivated on three different media, namely a rich (YEPD),a synthetic mineral (SMM) and a synthetic mineral enriched (SMM-AA) supplemented with amino acids leucine, isoleucine, valine and phenylalanine, each added at 1% (w/v), and higher alcohols and corresponding esters was determined at the time glucose was exhausted from the medium. As shown in Table 4, 2-methyl-2-butanol (amyl-alcohol, 2-MB), 3-methylbutanol (isoamyl alcohol, 3-MB) and 2-phenylethanol (2-PE) were identified in the fermentation broth of these yeasts. In a glucose-rich medium, *P. kudriavezii* species was about two times more efficient than the *S. cerevisiae* CEN.PK122-2N in producing the three different volatiles compounds, whereas *C. lusitaniae* exhibited roughly the same capacity as *S. cerevisiae*. In a glucose mineral medium, the amount of fusel alcohols was, as expected, extremely low, indicating that the production of these higher alcohols in a glucose rich medium was likely the result of the bioconversion of excess of branched-chain and aromatic amino acids present in this medium by the catabolic Ehrlich pathway (Hazelwood et al., 2008). Accordingly, the supplementation of the mineral medium with linear, branched and aromatic amino acids (leucine, isoleucine, valine and phenylalanine) boosted the production of these fusel alcohols, with a profile that was again yeast-dependent. Unlike *H. uvarum,* which was unable to grow in this medium, *C. lusitaniae* species showed a higher capacity than *S. cerevisiae* in the formation of the three flavours. Like on YEPD medium, *K. ohmeri* had the highest production of 3-MB but the lowest formation of 2-PE, when cultivated on SMM-AA. On the other hand, the flavours profile of *P. kudriavezii*on a synthetic medium supplemented with these amino acids was remarkably singular since the formation of 2-PE was strongly stimulated upon addition of phenylalanine, reaching a concentration that was 5 times higher than in *S. cerevisiae*, while levels 2-MB and 3-MB were significantly not different from those measured in YEPD.

This result suggest that the bioconversion of phenyl-lanine into 2-PE by the catabolic Erlich pathway is likely more efficient in *P. kudriavezii* than in *S. cerevisiae*. Thus, it would be worth to compare the enzymatic capacities of the Erlich pathway between these two yeast species, and in particular the amino transaminase and decarboxylase that play a key role in this pathway (Hazelwood et al., 2008). Moreover, the higher level of 2-PE produced by *P. kudriavezii* corroborated with the greater resistance of this yeast species to this aromatic compound. Finally, the corresponding esters of the higher alcohols (2-MB, 3-MB and 2-PE) were below detection in all of the five yeast species cultivated in SMM supple-mented with amino acids (data not shown).

Conclusions

In this work, we isolated nine yeast species from tradi-tional Algerian food and dairy products. Our analysis focused on five of these yeast isolates revealed important physiological and metabolic properties. In particular, it was found that *P. kudriavezii* was remarkably able to grow at higher temperature (>40°C), and much more tolerant to ethanol and osmotic pressure and was a better producer of 2-phenylethanol than *S. cerevisiae*. All these physiological traits may place this yeast species as an attractive alternative yeast platform in the field of bio-based production from renewable sources.

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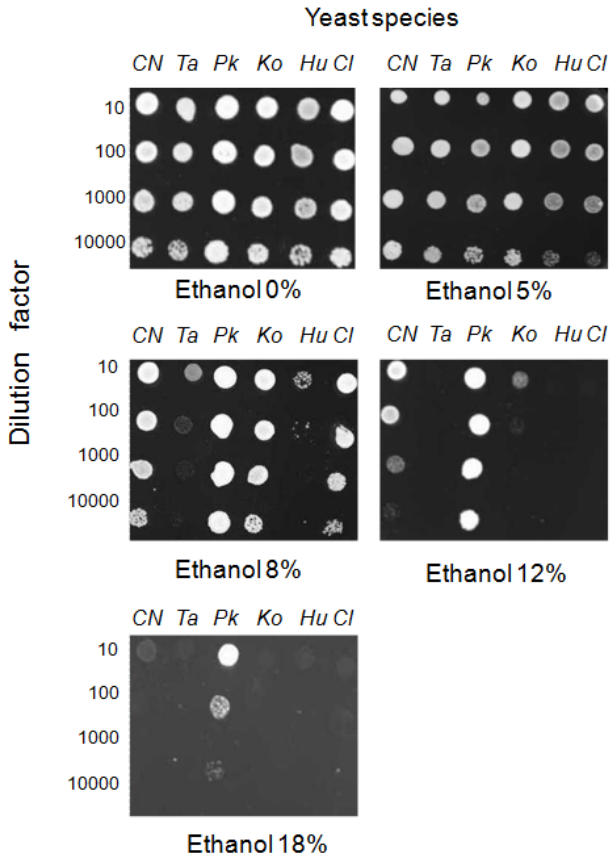

Figure 2. Effect of ethanol on the growth of yeast isolates on YEPD agar plates. Growth was scored after 2 days at 30°C. Abbreviations for yeast isolates are: *C.N* stands for *S cerevisiae* CEN.PK122-2N; Ta for *Trichosporon asahii*; Pk for *Pichia kudriavezii;* Ko for *Kodamaea ohmeri*; Hu for *Hanseniaspora uvarum* and Cl for *Clavispora lusitaniae*.

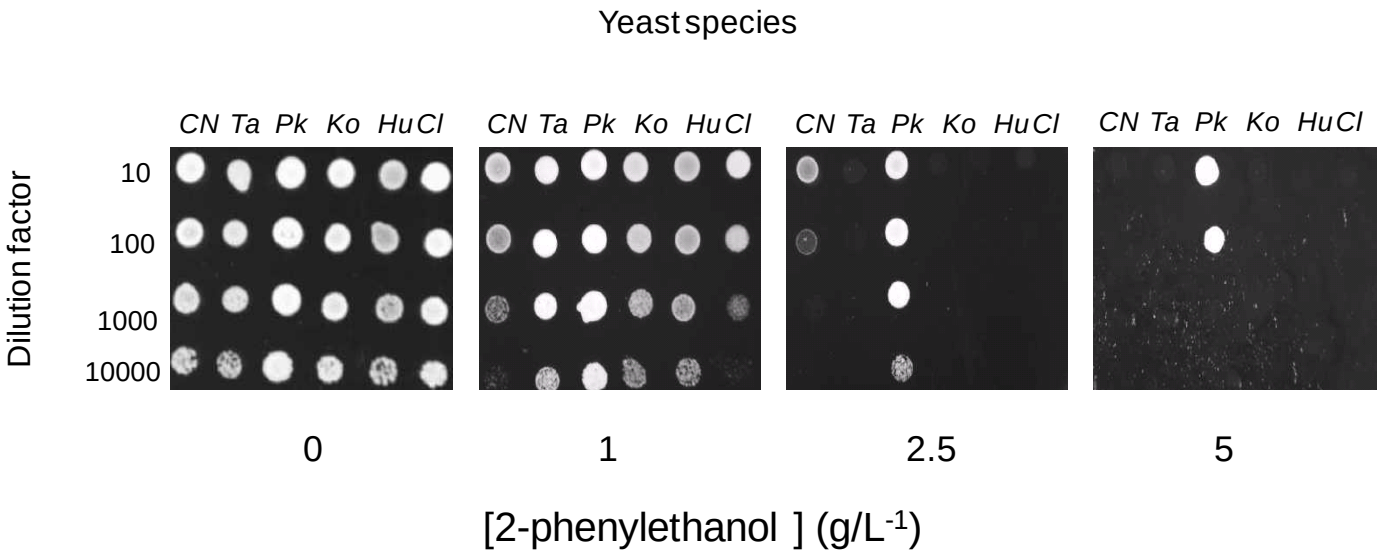

Figure 3. Effect of 2-phenyethanol on the growth of yeast isolates on YEPD agar plates. Growth was scored after 2 days at 30°C. Abbreviations are as in Figure 2.

Table 3. Flavours production by the different yeast isolates.

strain	YEPD (µmol/g dry mass)			SMM (µmol/g dry mass)			SMM + amino acids* (µmol/g dry mass)		
	2-MB	3-MB	2-PE	2-MB	3-MB	2PE	2-MB	3-MB	2-PE
S. cerevisiae CEN.PK122-2N	90	270	85	15	116	30	750	900	580
C. lusitaniae	80	137	60	25	108	60	880	1000	1600
P.kudriavezii	238	408	306	30	238	100	238	680	2857
H. uvarum[$]	82	578	85	-	-	-	-	-	-
K.ohmeri	288	638	20	85	320	20	800	2244	192
T. asahii	bd	bd	bd	bd	bd	bd	bd	bd	bd

The yeast isolates were cultivated on glucose-rich (YEPD) medium, glucose synthetic mineral medium (SMM) or supplemented with aromatic and branched chain amino acids (SMM + amino acids). Samples for analysis of flavours was taken at the end of growth when glucose was consumed. *Amino acids added to SMM were leucine, valine, isoleucine and phenylalanine at 1% (w/v); [$]No growth on SMM. Values are the mean of two independent experiments, with a standard deviation that was less than 15% between the two experiments. Abbreviations are: 2-MB; 2-methylbutanol or amyl alcohol; 3-MB; 3-methyl butanol or isoamyl alcohol; 2-PE, 2-phenylethanol; bd = below detection.

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