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ORIGINAL PAPER

Yeasts isolated from Algerian infants's feces revealed a burden of *Candida albicans* species, non-*albicans Candida* species and *Saccharomyces cerevisiae*

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Abstract This study aimed at showing the yeast diversity in feces of Algerian infants, aged between 1 and 24 months, hospitalized at Bejaia hospital (northeast side of the country). Thus, 20 colonies with yeast characteristics were isolated and identified using biochemical (ID32C Api system) and molecular (sequencing of ITS1-5.8S-ITS2 region) methods. Almost all colonies isolated (19 strains) were identified as *Candida* spp., with predominance of *Candida albicans* species, and one strain was identified as *Saccharomyces cerevisiae*. Screening of strains with inhibitory activities unveiled the potential of *Candida parapsilosis* P48L1 and *Candida albicans* P51L1 to inhibit the growth of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Further studies performed with these two *Candida* strains revealed their susceptibility to clinically used antifungal compounds and were then characterized for their cytotoxicity and hemolytic properties. On the other hand, *Saccharomyces cerevisiae* P9L1 isolated as well in this study was shown to be devoid of antagonism but resulted safe and overall usable as probiotic.

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Keywords Infant feces · *Candida* species · Antagonistic yeasts · *Saccharomyces cerevisiae* · Probiotic

Introduction

The human gastrointestinal tract is a complex ecological niche containing a multitude of microbes including bacteria, yeast and fungi. These microbes are foreseen to be inherited from the mother and the surrounding environments and colonize the gastrointestinal tract of infants immediately after the birth (O'Hara and Shanahan 2006). Bacteria are the most dominant microbes (more than 99 % of total population) (Ojetti et al. 2009), but eukaryotic cells such as yeasts and filamentous fungi are also part of this microbiota (Rajilić-Stojanović et al. 2009). The presence of yeasts in the gastrointestinal tract of infants was observed at early age, although these unicellular eukaryotic cells represent <1 % of the gut microbiota (Chandler et al. 1980). However, their role and functions in the equilibrium of the microbiota are not insignificant (Parfrey et al. 2011). *Candida* species are typically harmless eukaryotic commensal yeasts that are members of the Ascomycota phylum and can thus be recovered from the mammalian and environment sources. They are detected in all gastrointestinal sections of about 70 % of healthy adults and are normally found in feces. Most of yeasts isolated are members of the *Candida* genus (Schulze and Sonnenborn 2009) and are commonly located in the normal commensal microbiota on the mucosal surfaces of the gastrointestinal and genitourinary tracts (Kumamoto 2011). *Candida* species were also encountered in the majority of neonates by the end of the first month of life (Schulze and Sonnenborn 2009). Remarkably, *Candida* genus could provoke local or systemic opportunistic infections in hospitalized patients,

particularly in patients with compromised immune system (Moris et al. 2008). Most often *Candida albicans* is the guilty agent, but prevalence of non-*albicans Candida* species in some patients was reported (Mac Callum 2010). Harmless and nonpathogenic species such as *Saccharomyces cerevisiae* were also reported (Psomas et al. 2001). *S. cerevisiae* is marketed as probiotic formulation to treat various human gastrointestinal disorders (Lukaszewicz 2012). The beneficial effects of yeasts include their competitiveness for nutrients and adhesion, production of antagonistic compounds, immunomodulation, cholesterol assimilation, toxin elimination and adhesion and then neutralization of pathogenic bacteria (Moslehi-Jenabian et al. 2010; Hatoum et al. 2012; Ceugniez et al. 2015). Here, we use a culture-dependent approach to establish the yeast diversity of Algerian infants hospitalized at Bejaia hospital, for reasons other than gastrointestinal disorders. This analysis permitted the isolation of 20 yeasts with a bulk of *C. albicans* species, and lesser with non-*albicans Candida* species and one isolate of *S. cerevisiae* P9L1. This report unveils the inhibitory properties of yeast pathogens *C. parapsilosis* P48L1 and *C. albicans* P51L1. These antagonistic yeasts as well as *S. cerevisiae* P9L1 were deeply studied for their negative and positive effects. To the best of our knowledge, this is the first report shedding light and dealing with yeast diversity from an Algerian infants reservoir.

Materials and methods

Isolation of yeasts

Yeasts were isolated from feces of 62 infants without gastrointestinal disorders (diarrhea), aged between 1 and 24 months, and hospitalized in the pediatric service of Khalil Amrane hospital at Bejaia city (Algeria). An aliquot (1 g) of feces was resuspended in 9 ml of saline solution (0.9 % NaCl) and then plated in yeast extract-peptone-dextrose agar (YPD), pH 5.5, supplemented with 100 µg/ml of filter-sterilized oxytetracycline (Biokar, France). Plates were incubated at 37 °C for 48 h. Five colonies with yeast characteristics were purified by repeated streaking on YPD agar plates. These colonies were then maintained in YPD broth at +4 °C until use.

Biochemical identification of yeasts with ID 32C Api systems

All yeast colonies were identified based on their biochemical profiles using ID32C strips (BioMérieux, Marcy l'Etoile, France). Before inoculation of the ID32C system, strains were streaked on YPD agar plates and incubated for 24 h at 25 °C. After inoculation, ID32C systems were incubated at 25 °C for 48 h. After this period of incubation, the carbohydrate assimilation profile obtained for each tested isolate was compared to the database apiweb version 3.0 (BioMérieux) to obtain the final yeast diagnosis (Ceugniez et al. 2015).

Statistical analysis of ID32 C and ITS1-5.8S-ITS2 rDNA sequencing was performed with the software SPSS® Statistic v20 of IBM® (Armonk, NY, USA). Correlation and comparison were made with the crossed grid methods using Kappa of Cohen value.

Molecular identification of yeasts by sequencing their ITS1-5.8S-ITS regions

Total DNA was extracted from each isolate with the Bust n'Grab method (Harju et al. 2004). Afterward, it was quantified and amplified using the ITS1 forward primer 5′-TCC GTA GGT GAA CCT GCG G-3′ and the ITS4 reverse primer 5′-TCC TCC GCT TAT TGA TAT GC-3′ (Belloch et al. 1998; Schoch et al. 2012) and the following PCR program: initial denaturation 5 min at 94 °C, denaturation 30 s at 94 °C, annealing 30 s at 57 °C, elongation 1 min at 72 °C and final elongation 5 min at 72 °C. Thirty-five cycles were used for each amplification.

PCR products were purified using the Nucleospin® Gel and PCR Clean-Up kit of Machery-Nagel (Düren, Germany). PCR products were sequenced at Eurofins Genomics (Ebersberg, Germany). All these sequences were compared to those available on the NCBI database using a megablast research, and confirmed using the Ribosomal Database project on sequence match with default parameters except size placed at "<1200" parameter (Cole et al. 2014).

Screening of yeasts with inhibitory activities

The spot-on-lawn method was used to evaluate the antagonistic activity of yeast isolates against *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *Listeria innocua* CLIP 74915. An overnight culture of each yeast isolate was prepared in YPD broth at 37 °C and individually inoculated onto YPD agar plates by spot-inoculating 5 µl, leading to a zone of inhibition of 6 mm. The plates were incubated at 37 °C for 48 h. The growth in each plate was then overlaid with 10 ml of molten nutrient agar [0.7 % (w/v) agar, Fluka, Ireland] previously inoculated with the pathogen cultures (10^6 CFU/ml). The plates were incubated aerobically at 37 °C for 18 h and then examined for growth inhibition zones (Gotcheva et al. 2002).

Hemolytic activity

The hemolytic activity of yeasts was carried out as recently described by Kumar and Sharma (2013) but with slight

modifications. Thus, 10 µl of yeast culture was spotted onto YPD agar plates containing 5 % (v/v) sheep blood (Eurobio, Les Ulis, France). Plates were incubated at 37 °C for 48 h in aerobic conditions. After this period, a transparent/semitransparent zone around the spots was considered as positive hemolytic activity.

Acid and bile tolerance

The survival under conditions simulating those encountered in the gastrointestinal tract was investigated for antagonistic yeasts and *S. cerevisiae*. To test the strains for acid tolerance, yeasts from fresh (24 h) culture in YPD broth were centrifuged (5000*g,* 10 min) and the pellets obtained were washed twice with phosphate-buffered saline (PBS) and then resuspended, at 10^7 CFU/ml, in 5 ml of PBS adjusted to pH 1.5, 2 or 3. Samples were taken after 0, 1 and 3 h of incubation at 37 °C and plated onto YPD agar to determine the number of viable cells. Survival rate was determined by comparing the number of viable cells after incubation to the initial suspended cells number. To test the bile tolerance, 5 ml PBS containing 0.3, 0.5, 1 and 2 % (w/v) oxgall bile (Sigma-Aldrich, Steinheim, Germany) was inoculated with the yeast culture (10^6 CFU/ml) and then incubated at 37 °C for 4 h. After incubation, survival rate was determined as described for acid tolerance assays.

Antifungal susceptibility tests

Antagonistic yeasts as well as *S. cerevisiae* were tested for in vitro susceptibility to amphotericin, 5-fluorocytosin, fluconazole, voriconazole and caspofungin of clinical use using the CLSI BMD method (Clinical and standard, Institute). Minimum inhibitory concentration (MIC) assays were read after 24 h of incubation when tested against *Candida* spp., whereas the MIC endpoints for triazoles were read after 48 h when they were tested against non-*Candida* yeasts. In all instances, MICs were determined visually as the lowest concentration of drug that caused significant diminution of growth levels.

Adhesion and cytotoxicity assays performed on antagonistic yeasts

Cell line culture

The cell line used for the adhesion and cytotoxicity assays was the human colorectal adenocarcinoma Caco-2 (Sigma-Aldrich, Steinheim, Germany). Cells were routinely grown in 75 cm^2 flasks in a controlled atmosphere of 5 % CO_2 and 95 % air at 37 °C in Dulbecco's Modified Essential Medium (DMEM, Biowest, France) containing 4.5 g/l glucose, 2 mM L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, 1 % (v/v) nonessential amino acids and 10 % (v/v) fetal bovine serum (FBS). All the reagents were from PAN-Biotech GmbH (Aidenbach, Germany).

Adhesion assay

To assess the adhesion potential, when sub-confluence was reached, Caco-2 cells were prepared in 24-well tissue culture plates by seeding 4×10^4 cells per well and incubating for 24 h. Overnight yeast cultures (10^7 CFU/ml) were harvested by centrifugation (5000*g,* 10 min), resuspended in DMEM without FBS and antibiotics and then applied on confluent Caco-2 cell monolayers (10^6 CFU/well). After 2 h of incubation at 37 °C (5 % CO_2 and 95 % air), monolayers were washed twice with 300 µl of phosphate-buffered saline (PBS, 100 mM, pH 7.4) to remove nonadherent yeasts, and Caco-2 cells were lyzed by incubation at room temperature for 15 min in the presence of 0.1 % Triton X100, to keep yeast only. Lysates were diluted and plated onto YPD agar to determine the number of adherent yeasts.

CCK-8 cytotoxicity assay

Cytotoxicity of selected yeast strains toward Caco-2 cells was assessed in vitro using the Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Japan). CCK-8 allows convenient assays using water-soluble tetrazolium salt: WST-8[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt)], which produces, upon bioreduction by cellular dehydrogenases, an orange-colored formazan. The amount of formazan produced is directly proportional to the number of living cells. Caco-2 cells were seeded at a density of 8×10^3 cells/well in 96-well cell culture plates and pre-incubated for 24 h before being incubated with yeasts (10^5 CFU/well) prepared in DMEM as described for the adhesion assay. After incubation, the medium was removed and cells were incubated with 150 µl of DMEM containing 50 µl of CCK-8 reagent for 4 h 37 °C. The relative viability (%) was then calculated based on absorbance at 450 nm using a microplate reader (Xenius SAFAS, Monaco). Results were expressed as percentage of proliferation. Viability of nontreated control cells was defined as 100 %. Absorbance of wells without Caco-2 cells and with yeasts was subtracted from absorbance of wells containing Caco-2 cells and yeasts.

Aggregation assays

The auto-aggregation assays were performed using the method described by Kos et al. (2003). Yeast was grown

for 24 h at 37 °C in YPD broth. The cells were harvested by centrifugation (5000*g,* 10 min at 4 °C), washed twice with PBS (pH 7.2) and resuspended in the PBS. Cells suspensions were mixed by vortexing for 30 s, and auto-aggregation was determined after 2 and 4 h of incubation at 37 °C. An aliquot (1 ml) of these suspensions was carefully removed from the upper zone, and the absorbance at 600 nm was read on a spectrophotometer (Shimadzu, Germany). The auto-aggregation percentage was expressed as:

$$1 - (A_t/A_0) \times 100,$$

where A_t represented the absorbance at time $t = 2$ or 4 h and A_0 the absorbance at $t = 0$ h.

For co-aggregation with pathogens, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were grown in nutrient broth at 37 °C for 18 h. Yeast and pathogens cell suspensions from overnight cultures were prepared as described above in PBS. Equal volumes (2 ml) of yeast and pathogen suspensions were mixed by vortexing 30 s in glass test tubes. Control tubes contained 4 ml of suspension of each bacterial and yeast strains. Absorbance was measured immediately and after 2 h incubation at 37 °C. The percentage of co-aggregation was calculated using equation:

$$\%\ \text{Co-aggregation} = \frac{(Ax + Ay)/2 - A(x + y)}{(Ax + Ay)/2} \times 100$$

where A represented absorbance, x and y represented each of the two strains in the control tubes, and $(x + y)$ their mixture.

Construction of phylogenetic tree

A phylogenetic tree was established on PhyML v3.1 based on Maximum-Likelihood algorithm, after ClustalΩ multialign of all sequenced strains, and with following parameters: bootstrap on branch support of 1000 replicates, nucleotide equilibrium frequencies on optimized, invariable sites in optimized, across site variations in optimized, tree-searching operation in best of NNI & SPR and starting tree at BioNJ and optimized tree topology. To set up this phylogenetic tree, we used the recent seaview software (Gouy et al. 2010).

Results

Yeasts isolated from Algerian infants display inhibitory properties

Yeasts were isolated from feces of infants aging between 1 and 24 months (Table 1). Afterward, their identification was performed by ID 32C Api system, and sequencing of the ITS1-5.8S-ITS2 region is shown in Table 1. Out of 20 strains identified by these two independent methods, only

Table 1 Identification of yeasts isolated from infant feces based on their phenotypic criteria (ID32C Api system) and sequences of the ITS1-5.8S-ITS2 region

Isolate	Infant	Age month	Sex	ITS1-5.8S-ITS2 sequencing	% ID	*e* value	ID 32C Api system	Reliability
P1L1	1	1	M	*Pichia kudriavzevii*	99	0	*Candida krusei*	Ok
P2L1	2	24	M	*Candida albicans*	99	7.00E−159	*Candida albicans*	Ok
P3L1	3	1	F	*Candida glabrata*	99	0	*Candida glabrata*	Ok
P5L1	5	2	M	*Candida glabrata*	99	0	*Candida albicans*	
P8L1	8	1	F	–			*Candida rugosa*	–
P9L1	9	24	M	*Saccharomyces cerevisiae*	100	6.00E−03	*Saccharomyces cerevisiae*	Ok
P11L1	11	15	M	*Candida parapsilosis*	100	0	*Candida parapsilosis*	Ok
P15L1	15	1	M	*Candida albicans*	99	0	*Candida albicans*	Ok
P18L1	18	1	F	*Candida albicans*	99	0	*Candida albicans*	Ok
P21L1	21	2	M	*Candida albicans*	99	0	*Candida albicans*	Ok
P25L1	25	2	M				*Candida tropicalis*	–
P27L1	27	6	M	*Candida tropicalis*	100	2.00E−102	*Candida parapsilosis*	
P35L1	35	2	M	*Candida albicans*	99	0	*Candida albicans*	Ok
P45L1	45	3	F	*Candida glabrata*	99	0	*Candida glabrata*	Ok
P48L1	48	12	F	*Candida parapsilosis*	100	0	*Candida parapsilosis*	Ok
P49L1	49	20	F	*Candida albicans*	99	0	*Candida albicans*	Ok
P51L1	51	24	M	*Candida albicans*	100	0	*Candida albicans*	Ok
P54L1	54	9	F	*Candida albicans*	99	0	*Candida albicans*	Ok
P56L1	56	2	M	*Candida rugosa*	99	4.00E−52	*Candida rugosa*	Ok
P62L1	62	20	M	*Clavispora lusitaniae*	100	2.00E−08	*Candida famata*	–
ATCC 10231				*Candida albicans*	100	0	–	Ok

the identification of P5L1 and P27L1 resulted to be different. The identification of P1L1 is consistent because *Pichia kuriavzevii*, which is a synonym for *Issatchenkia orientalis*, is the teleomorph of *C. krusei* (Table 1). Thus, these two independent methods showed a good correlation and concordance, as supported statistically with Kramer and Kappa values of 0.914 and 0.615, respectively. Out 20 colonies, 19 colonies belonged to *Candida* species, whilst one colony was identified as *S. cerevisiae* P9L1. In this repertoire, *C. albicans* was the dominant species. The ITS1-5.8S-ITS2 sequences of *C. albicans* isolated here were highly similar (>99 %) to that of *C. albicans* ATCC 10231 (Table 1). The phylogenetic tree indicated that all the *C. albicans* isolates belonged to the same group (Fig. 1; Table 2), considering the strain P35L1 as the most "ancestral". Remarkably, the reference strain *C. albicans* ATCC 10231, not present in the hospital, was in the

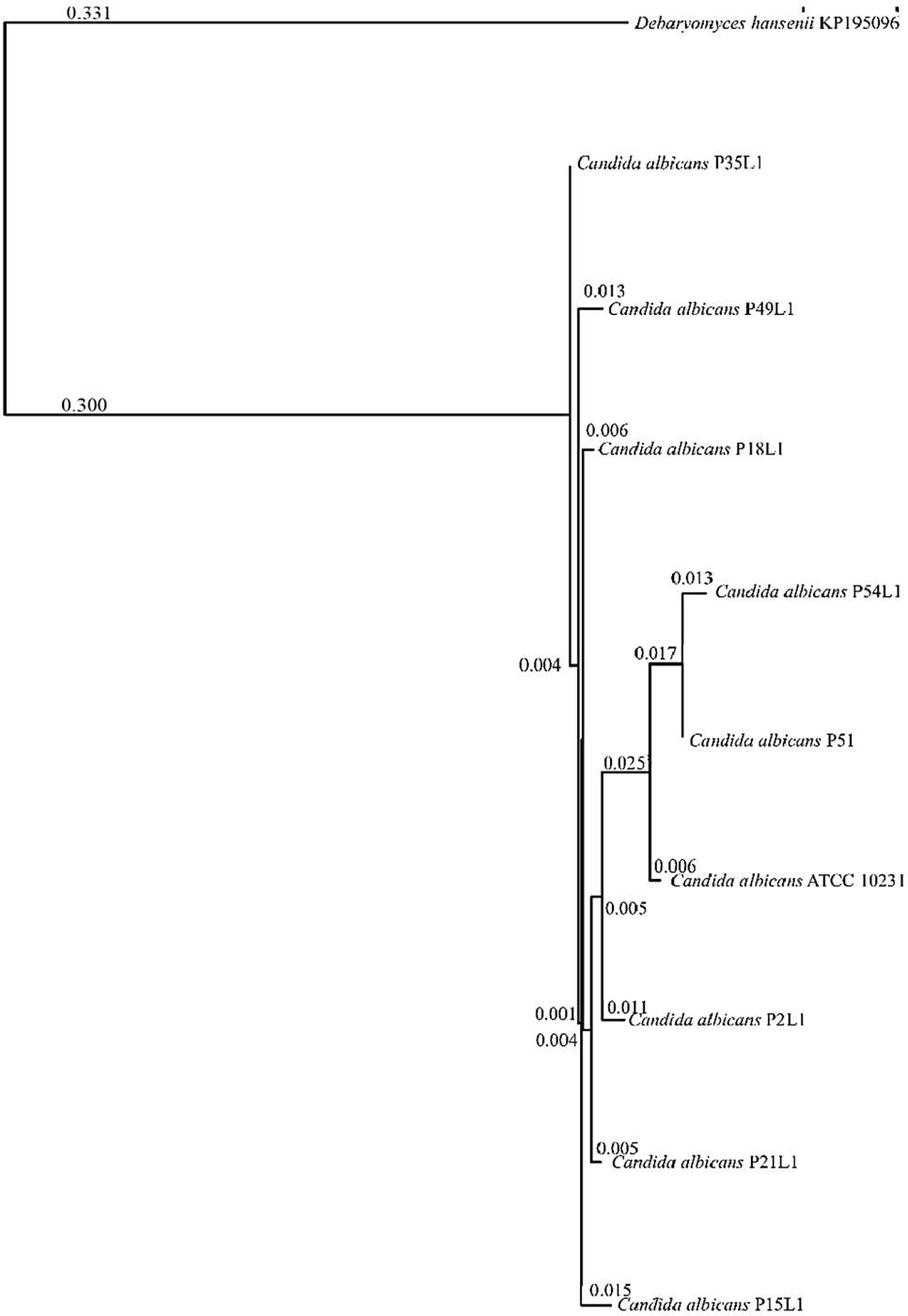

Fig. 1 Phylogenic tree based on 5.8S ITS1–ITS2 rDNA sequencing of species isolated from the feces of Algerian infants. The tree was established on maximum-likelihood analysis with a bootstrap on branch support of 1000 replicates

Table 2 Analysis of ITS1-5.8S-ITS2 similarities of *Candida albicans* species isolates

Similarity (%)	*D. hansenii* KP195096 (%)	*C. albicans* ATCC 102321 (%)	*C. albicans* P2L1 (%)	*C. albicans* P15L1 (%)	*C. albicans* P18L1 (%)	*C. albicans* P21L1 (%)	*C. albicans* P35L1 (%)	*C. albicans* P49L1 (%)	*C. albicans* P51L1 (%)	*C. albicans* P54L1 (%)
D. hansenii KP195096	100.0									
C. albicans ATCC 102321	96.0	100.0								
C. albicans P2L1	95.0	99.4	100.0							
C. albicans P15L1	95.2	98.6	99.0	100.0						
C. albicans P18L1	96.0	99.4	98.7	98.8	100.0					
C. albicans P21L1	96.0	99.4	99.4	98.6	100.0	100.0				
C. albicans P35L1	96.0	99.4	99.4	98.8	99.6	99.6	100.0			
C. albicans P49L1	96.0	99.4	99.4	98.6	99.8	99.8	99.8	100.0		
C. albicans P51L1	96.0	99.8	99.4	99.0	99.2	99.2	99.4	99.2	100.0	
C. albicans P54L1	95.9	99.5	99.5	98.7	99.2	99.2	99.2	99.2	99.7	100.0
Score with external taxon	98.6	Standard deviation with external taxon		1.5						
Score without external taxon	99.3	Standard deviation without external taxon		0.4						
Score without reference strain	99.3	Standard deviation without reference strain		0.3						

Table 3 Antagonism exerted by *Candida parapsilosis* P48L1, *Candida albicans* P51L1 and *Saccharomyces cerevisiae* P9L1

Yeast strain	Inhibitory activity (mm)		
	E. coli ATCC 25922	*S. aureus* ATCC 25923	*Listeria innocua* CLIP 74915
C. parapsilosis P48L1	14 ± 0.57	14 ± 1.00	0
C. albicans P51L1	12 ± 1.15	12 ± 0.57	0
S. cerevisiae P9L1	0	0	0

The data (±SD) are the mean of at least three independent experiments

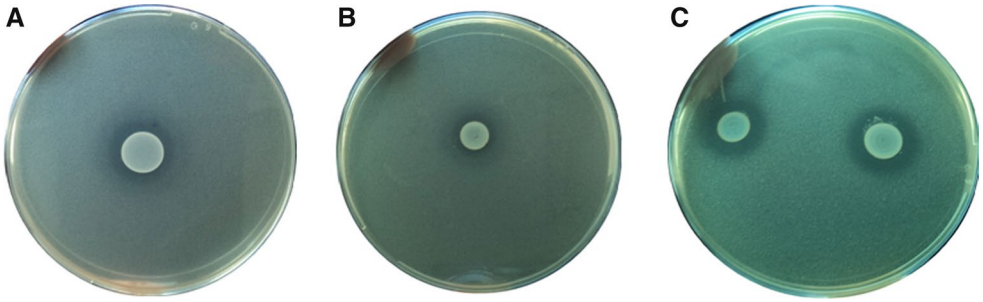

Fig. 2 Plates showing zones of growth inhibition of **a** *C. albicans* P51L1 against *E. coli* ATCC 25922, **b** *C. parapsilosis* P48L1 against *E. coli* ATCC 25922, **c** *C. parapsilosis* P48L1 and *C. albicans* P51L1 against *S. aureus* ATCC 25923

same group of hospital strains, advocating that a multigenic phylogenetic tree would be more suitable.

Furthermore, pathogen yeasts *C. parapsilosis* P48L1 and *C. albicans* P51L1 displayed inhibitory activities against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (Table 3; Fig. 2).

Highlights on probiotic properties of antagonistic yeasts

Adhesion and cytotoxicity to Caco-2 cells

The capabilities of adhesion of *C. parapsilosis* p48L1, *C. albicans* p51L1 and *S. cerevisiae* P9L1 to human Caco-2 cells are studied. As shown in Fig. 3a, the highest level of adhesion was obtained with *S. cerevisiae* P9L1 with 6.34 %, followed then by *C. parapsilosis* P48L1 with 2.03 % and *C. albicans* P51L1 with 0.76 %. Besides, a cytotoxic effect was observed as expected for *C. parapsilosis* P48L1 and *C. albicans* P51L1 with 5.33 and 20.56 % survival, respectively (Fig. 3b). However, no cytotoxic effect was registered for *S. cerevisiae* P9L1 (Fig. 3b), as no significant difference was noted between the control test (noninfected Caco-2 cells) and *S. cerevisiae* P9L1 with a p value >0.5.

Resistance to acidic conditions, bile concentration and antifungal compounds

As shown on Fig. 4a, the survival of *S. cerevisiae* P9L1 was pH dependent. At pH 3, this yeast resisted perfectly even after 3 h of incubation. When the pH was adjusted to 2 and 1.5, a drop in survival rate was registered reaching thereof 92.1 and 28.4 % after 3 h of incubation, respectively. Conversely, no decrease in survival of *C. parapsilosis* P48L1 and *C. albicans* P51L1 was registered. Similarly, these three yeasts were examined for their bile tolerance. As shown on Fig. 4b, *C. parapsilosis* P48L1 and *C. albicans* P51L1 were fully resistant to 2 % (not for 4 %, data not shown) of bile concentration and after 4 h of incubation, contrarily to *S. cerevisiae* P9L1, which was unable to support such harsh conditions as only 46.7 % of survival was registered.

Moreover, the antifungal susceptibility profiles of *C. parapsilosis* P48L1, *C. albicans* P51L1 and *S. cerevisiae* P9L1 were performed in an effort to provide MIC data (Table 4) for optimal therapy. The assays performed showed that *C. parapsilosis* P48L1, *C. albicans* P51L1 and *S. cerevisiae* P9L1 were susceptible to clinical antifungal compounds such as amphotericin B, 5-fluorocytosin, fluonazole, voriconazole and caspofungin. The interpretation of susceptibility and resistance to antifungal compounds were performed in agreement with the EUCAST and CLSI recommendations (Cuenca-Estrella et al. 2012).

Hemolytic activities and aggregation properties

Candida albicans P51L1 and *S. cerevisiae* P9L1 could be considered as nonhemolytic strains contrary to *C. parapsilosis* P48L1 which resulted to be hemolytic (Fig. 5). The auto-aggregation rate of *S. cerevisiae* was 22.5 ± 2.6 and 45.9 ± 3.4 % after 2 and 4 h of incubation, respectively.

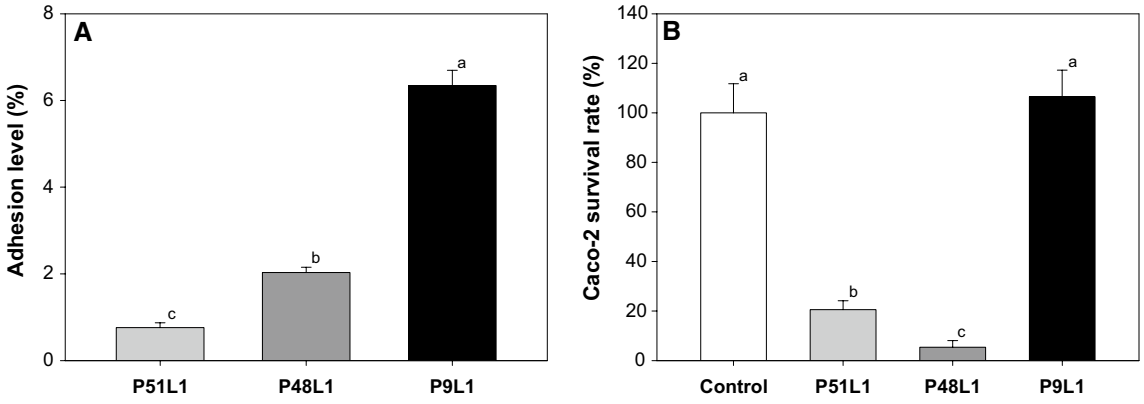

Fig. 3 **a** Adhesion capabilities of *C. albicans* P51L1, *C. parapsilosis* P48L1 and *S. cerevisiae* P9L1 to Caco-2 cells after 2 h of incubation at 37 °C. The data are the means of at least three independent experiments. **b** Cytotoxic effect of *C. albicans* P51L1, *C. parapsilosis* P48L1 and *S. cerevisiae* P9L1 on Caco-2 cells (24 h). The data are the means of at least three independent experiments. Means without a *common letter* are different ($p < 0.01$) using one-way ANOVA with Tukey test for pairwise comparisons

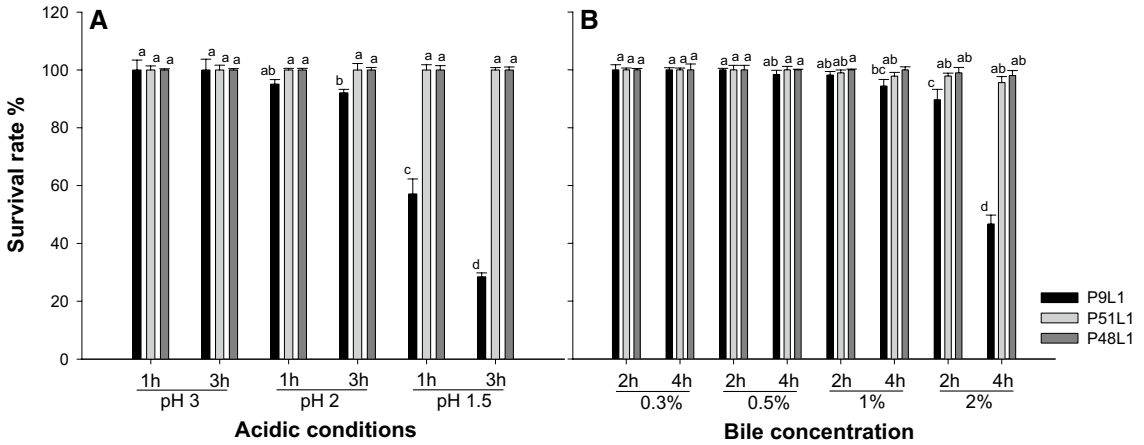

Fig. 4 **a** Survival rate of the strains *S. cerevisiae* P9L1, *C. parapsilosis* P48L1 and *C. albicans* P51L1 under acidic pH conditions after 1 and 3 h of incubation. **b** Survival rate of *S. cerevisiae* P9L1, *C. parapsilosis* P48L1 and *C. albicans* P51L1 at different bile concentrations after 2 and 4 h of incubation. The standard deviations are indicated by *error bars*. The data are the means of values from three independent experiments. Means without a *common letter* are different ($p < 0.05$) using one-way ANOVA with Tukey test for pairwise comparisons

Table 4 Antifungal susceptibility tests of antagonistic yeasts and *S. cerevisiae* P911 isolated in this work

Clinical antifungal susceptibility tests	*S. cerevisiae* P911 MIC (µg/ml)	*C. parapsilosis* P48L1 MIC (µg/ml)	*C. albicans* ATCC 10231 MIC (µg/ml)
Amphotericin	0.75 (S)	0.500 (S)	0.380 (S)
5 Fluorocytosin	0.012 (S)	0.023 (S)	0.064 (S)
Fluconazole	3.00 (S)	0.190 (S)	0.750 (S)
Voriconazole	0.032 (S)	0.120 (S)	0.016 (S)
Caspofungin	0.190 (S)	0.380 (R)	0.016 (S)

Susceptibility (S) and resistance (R) were determined according to EUCAST and CLSI recommendations (Cuenca-Estrella et al. 2012)

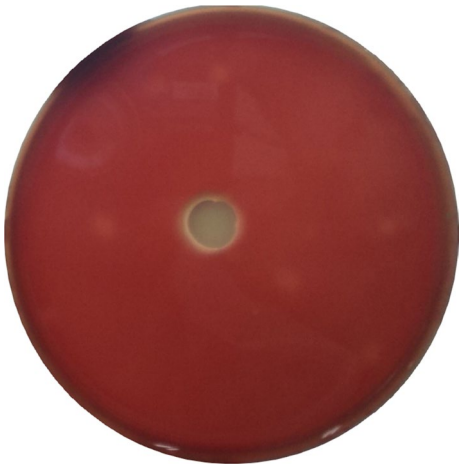

Fig. 5 Plates showing alpha-hemolysis of *C. parapsilosis* P48L1

This strain co-aggregated with two tested pathogenic bacteria. Notably, the co-aggregation was stronger and faster with *E. coli* ATCC 25922 (38.2 ± 3.9 %) than with *S. aureus* ATCC 25923 (23.5 ± 2.9 %) after 2 h of incubation. Conversely, the antagonistic strains *C. parapsilosis* P48L1 and *C. albicans* P51L1 did not co-aggregate with the tested pathogenic bacteria. These strains showed an auto-aggregation rate of 41.5 ± 1.9 and 31.6 ± 2.9 after 4 h, respectively (Table 5). Thus, *C. albicans* P51L1 was the less auto-aggregative strain.

Accession numbers

The ITS1-5.8S-ITS2 rDNA sequences of the strains isolated here are available on NCBI under accession number KP878240 to KP878254.

Discussion

This study showed, for the first time, the yeast diversity of Algerian infants hospitalized at Bejaia hospital. Out of 20 strains isolated and purified from feces of different donors, 19 strains belonged to *Candida* species, and one was identified as *S. cerevisiae* P9L1. The identification performed with biochemical (ID32C Api system) and molecular (sequencing of ITS1-5.8S-ITS2 region) methods showed a very good reliability. The most abundant species recovered was *C. albicans*, a species that has been found in diverse niches including the normal gastrointestinal tract, oral

Table 5 Auto-aggregation and co-aggregation scores of yeasts isolated in this study

Time (h)/pathogenic bacteria	Auto-aggregation (%)		Co-aggregation (%)	
	2 h	4 h	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923
P1L1	3.1 ± 1.55	9 ± 1.7	–	–
P2L1	6.1 ± 2.1	18.3 ± 2.73	–	–
P3L1	8.3 ± 2	17.7 ± 2.6	–	–
P5L1	2.7 ± 2	12.9 ± 2.5	–	–
P5L2	12.6 ± 3.5	27.4 ± 2.4	–	–
P8L1	5.1 ± 1.73	14.5 ± 2.6	–	–
P9L1	22. ± 2.6	45.9 ± 3.4	38.2 ± 3.9	23.5 ± 2.9
P1Ll1	13.5 ± 2	42.2 ± 2.4	29	–
P15L1	8.5 ± 2	22 ± 2.7	–	–
P18L1	9.8 ± 1.2	25.8 ± 2.8	–	–
P21L1	15.2 ± 3.1	45.5 ± 2.4	31.3	13.1
P25L1	13.9 ± 2.5	36.6 ± 2.7	–	–
P27L1	11.6 ± 3.2	25.4 ± 3.3	–	–
P35L1	5.6 ± 1.8	18.2 ± 2.1	–	–
P45L1	16 ± 2.1	36 ± 3.2	–	–
P48L1	18.4 ± 2.1	41.5 ± 1.9	–	–
P49L1	4.2 ± 2.1	12.2 ± 2.25	–	–
P51L1	13.6 ± 1.9	31.3 ± 3.9	–	–
P54L1	9.6 ± 1.3	25.9 ± 2.1	–	–
P55L1	7.8 ± 1.3	19.2 ± 2	–	–
P62L1	5.4 ± 1	13.3 ± 2.8	–	–

– No co-aggregation was observed

cavity and urogenital tract microbiota (De Vos et al. 2012). Recently, Papon et al. (2013) pointed out *C. albicans* as the most frequent agent of candidiasis. Similar studies carried out worldwide reported the dominance of *C. albicans* species in infant feces. Indeed, Rozkiewicz et al. (2005) studied the prevalence of this pathogen in feces of hospitalized children with and without diarrhea in Bialystok (Poland). The authors identified *C. parapsilosis* and *C. krusei*, and noteworthy *C. albicans*. Indeed, 131 strains belonged to *C. albicans* (Rozkiewicz et al. 2005). The incidence of fecal *C. albicans* in children with and without diarrhea was comparable (Rozkiewicz et al. 2005). In another study carried out on Nigerian infants, *C. albicans* resulted as the dominant species even other species including *C. tropicalis, C. pseudotropicalis, C. glabrata* and *C. parapsilosis* were also identified (Enweani et al. 1994). The study realized by Forbes et al. (2001) on fecal yeasts in feces of hospitalized children in Western Australia has revealed the presence of *C. albicans*, *C. parapsilosis*, *C. guillermondii*, *C. lipolytica*, *C. humicola, Torulopsis glabrata, Trichsporon beigelii* and *Rhodotorula glutinis,* but *C. albicans* was the most commonly identified species.

Moreover, non-*albicans Candida* (NAC) species have been also isolated from feces of Algerian infants but with a lower isolation rate. The NAC species isolated were *C. krusei* (synonym: *Issatchenkia orientalis*), *C. glabrata, C. rugosa, C. parapsilosis, C. tropicalis, C. rugosa* and *C. famata* (synonym: *Debaryomyces hansenii*). According to Papon et al. (2013), the NAC species now account for a substantial part of clinical isolates collected worldwide in hospitals.

The last decade has demonstrated the success of DNA-sequence-based methods (SBM methods) as suitable method to study epidemiology of *C. albicans*. To strengthen the SBM approaches, we show here that ITS1-5.8S-ITS2 sequences analysis is a robust and affordable method that might be used to identify *C. albicans* species and NAC species. The dendrogram resulting from ITS1-5.8S-ITS2 sequences analysis led us to think that our *C. albicans* isolates are genetically related to *C. albicans* ATCC 10231 (data not shown).

Screening of inhibitory activities has shed light on the potential of *C. parapsilosis* P48L1 and *C. albicans* P51L1 to inhibit *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. In the best of our knowledge, this is the first report underlining inhibitory properties of notorious pathogen yeast species from infant origin. *C. parapsilosis* P48L1 and *C. albicans* P51L1 were active against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 although they were unable to co-aggregate and weakly aggregating, arguing that a cell–cell contact might not be only the inhibitory pathway requested. Related to inhibitory activities of yeasts, Hatoum et al. (2012) reported that most often yeast antagonism was attributed to killer toxins. However, yeast antagonism remains to be investigated contrarily to its bacterial counterpart, which is well characterized (Drider and Rebuffat 2011). The auto-aggregation is considered as key element for adhesion and biofilm formation. Indeed, the auto-aggregation potential reported for *S. cerevisiae* P9L1 renders this strain as potential candidate for probiotic design. Moreover, the co-aggregation confers the ability to form a barrier against pathogens, and it is thereof considered as an important property for probiotic strains according to Mobili et al. (2010). *S. cerevisiae* P9L1 isolated in this study fulfills these properties.

The adverse effects registered for *C. parapsilosis* P48L1 and *C. albicans* P51L1 yeasts render evidently these strains as nonsuitable for probiotic design in spite of their antagonism against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, their in vitro tolerance to bile salts and susceptibility to traditional antifungal compounds such as amphotericin B, 5-fluorocytosin, fluonazole, voriconazole and caspofungin, which are routinely and clinically used and recommended to treat fungi infections therapy.

Further, *C. parapsilosis* P48L1 and *C. albicans* P51L1, conversely to harmless *S. cerevisiae* P9L1, displayed low adhesion score and cytotoxic effects to human epithelial colorectal adenocarcinoma Caco-2 cells. The adhesion process to host cells has been described as a prerequisite factor in the *Candida* infection process (Schulze and Sonnenborn 2009; Vazquez and Sobel 2011) on the other hand considered the adhesion step as a key event, which permits interactions between probiotics and the host cells (Lu and Walker 2001).

To sum up, this prospective study allowed the isolation of 20 yeasts including *C. albicans* and NAC species from Algerian infants. *C. parapsilosis* P48L1 and *C. albicans* P51L1 exhibited antibacterial activity against pathogenic bacteria but resulted to be toxic. *S. cerevisiae* P9L1 appeared to be devoid of hemolytic activity, resistant to acidic conditions, tolerant to increasing bile concentration, and able to adhere to epithelial cells, aggregate and co-aggregates with pathogens. To the best of our knowledge, this is the first report discussing yeast diversity in a north-African reservoir and characterizing these yeasts for their positive and negative effects.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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