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ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF SOME LACTOBACILLI ISOLATED FROM CAMEL'S MILK BIOTOPE IN THE SOUTH OF ALGERIA

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ABSTRACT

Antifungal activity has been the subject of numerous recent works to fight the development of molds in food products and to reduce alteration. The objective of this study is the detection of new indigenous lactic acid bacteria (LAB) strains isolated from Algerian camel's milk which have an antifungal activity. A number of 264 LAB were isolated from fermented camel's milk where 80 rods isolates were identified by phenotypical, physiological and biochemical tests. All retained isolates belong to the genus *Lactobacillus* (Lb.). The antifungal activity was determined by using confrontation and overlay techniques. The screening of antifungal activity against *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium* sp. showed that 14 strains have an antifungal activity. These later isolates were identified as *Lb. plantrarum, Lb. paracasei subsp. paracasei, Lb. brevis, Lb. rhamnosus* and *Lb. pentosus*. These strains have shown an effect on mycelial growth and spore germination. *Penicillium sp*. is the most inhibited followed by *Aspergillus* ssp. No inhibitory activity could be detected against fungi with the supernatant. However, supernatant have an inhibitory activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Listeria ivanovii*. *P.* aeruginosa is the only specie who has been affected by the neutralized supernatant. The redissolved dry supernatant of *Lb. brevis* revealed an inhibition of both fungal and bacterial indicator strains. Results suggest that camel's milk LAB's strains could be selected for application to control spoilage, fungal growth and pathogenic bacteria. The use of LAB producing antifungal metabolites represents a technological advantage for the food industry.

Keywords: Antagonism, Antifungal activity, Antimicrobial, Biopreservation, Camel's milk, *Lactobacillus*

INTRODUCTION

Lactic acid bacteria (LAB) play an essential role in many technological processes, and especially in fermentation of food products. LAB was associated with several foods of animal or plant origin such as dairy products, meats, bakery products and also animal feed as silage. These LAB have GRAS status in the United States which means "generally recognized as safe" **(Pawlowska et *al*., 2012).** Molds are the most microorganisms implicated in the spoilage of foods and crops and causing a loss of 5 to 10% of global production **(Ström, 2005).** Mycotoxins are produced on a wide variety of food, before, during and after harvest. They affect many agricultural products including cereals, dried fruits, nuts, coffee beans, grapes and oilseeds. Mycotoxins are considered a part of the most significant food contaminants in terms of impact on public health, food security and the economies of some countries **(Steyn, 1995; Pitt, 2000)**. From hundreds of mycotoxins identified at present, thirty are of significant effects in human and animal health because of their frequency or toxicity **(Bennett et Klich, 2003).** Chemical and physical control methods against phytopathogenic microorganisms exist, but, they are not always satisfactory. The use of chemicals such as feed additives or biocides in agriculture has increased in recent years, despite their effectiveness and preservative effect, however, they remain highly toxic and carcinogenic **(Ashley-Martin et *al*., 2012)**.
LAB are known as agents of food preservation, this preservative effect is related to the production of various inhibitory metabolites such as organic acids, hydrogen peroxide, bacteriocins, hydroxylated, fatty acids, diacetyl and reuterin **(Dalié et *al*., 2010; Russo et *al*., 2017)**. Currently it has become imperative to find new LAB strains, able to be used as biopreservative agents to minimize losses in food industry.
The objective of our study was the selection of bacterial strains belonging to the genus *Lactobacillus* isolated from Algerian camel's milk, to study their ability to inhibit the mycelial growth and conidia germination of some fungi and to show the spectrum activity of their metabolites against some bacterial strains also.

MATERIAL AND METHODS

Sampling

Camel's milk was collected from 8 sites located in the following regions in Algeria: El Abadla, Oran, Tabelbala, Adrar, Mecheria, Beni-Abbès, Timimoun and Ghardaïa. Samples were collected in sterilized bottles, kept in cool box (<10°C) containing ice packs during the transport to laboratory and processed within 2 days. The milk samples were incubated for 48 h at 30°C until the milk being coagulated. This incubation stimulates the growth of LAB present in raw milk.

Isolation of lactic acid bacteria

For the isolation of LAB, successive decimal dilutions were performed in sterile physiological water to the order of 10^{-7}. A volume of 1ml was taken from the dilution 10^{-5}, 10^{-6} and inoculated in selective MRS medium supplemented with $CaCO_3$ (5g/l) (Sigma-Aldrich, Germany) to reveal production of lactic acid by a clear zone around the colonies and 0.01% cycloheximide (Sigma-Aldrich, Germany) to inhibit yeast and fungal growth. Cultures were incubated anaerobically at 30°C for 72 h. After incubation, ten (10) representative and typical colonies of LAB (shape, size, pigmentation, outline, viscosity) were randomly chosen.

Purification of isolates

The selected colonies were then sub-cultured in MRS broth and incubated at 30°C. After incubation, the strains that have developed were inoculated successively on MRS medium supplemented with 0.01% of cycloheximide. Isolates have been examined for the Gram stain and catalase reaction. Gram positive and catalase negative isolates were presumed as LAB. The strains of LAB were stored in skimmed milk with 30% (v/v) glycerol (Biolife, Italy) at -20°C.

LAB identification

Temperature growth was assessed by the presence of an unclear MRS broth culture after 72 h of incubation at 15, 37 and 45°C. The fermentation type was determined by gas production from glucose in MRS broth, containing inverted Durham tube. To determine the fermentation type, sodium-gluconate-MRS broth was used. Presence of arginine dihydrolase (ADH) was tested on M16-BCP medium. LAB which metabolizes lactose, produce lactic acid and acidify the medium then give a yellow coloration around the colonies. While for those were able to use arginine, the release of NH_4 re-alkalize the medium then colonies appear white **(Thomas, 1973; Mathot et *al.*, 1994).**

Carbohydrates Fermentation profile

The carbohydrates API 50 CHL test kit (bioMérieux, France) were used to test the ability of isolates to ferment 49 carbohydrates. A fresh culture (18-24 h) in MRS broth was centrifuged and washed with physiological water. The cells were introduced into API CHL medium. These samples were then tested with the API strips according to the manufacturer's instructions, cupules were covered with paraffin oil, and the results were read after 24 h and 48 h of incubation at 30°C. The species identification was established with apiweb™ V5.2 (bioMérieux).

Origin of fungal and bacterial strains

Aspergillus niger was procured from parasitology and mycology laboratory of Sidi Bel Abbes hospital. The strain *Penicillium* sp. obtained from Mascara hospital and *Aspergillus fumigatus* MNHN566 from the National Museum of Natural History, France. The bacterial strains, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, were offered by the central laboratory of Oran hospital. *Listeria ivanovii* ATCC 19119 comes from the collection of Applied Microbiology Laboratory Oran 1 University. *Pseudomonas aeruginosa* is belonging to the collection of Environmental Surveillance Network Laboratory Oran 1 University.

Fungal growth conditions

The three strains *A. niger, A. fumigatus* and *Penicillium.* sp were grown on potato dextrose agar (PDA) medium at 30°C for 7 days and then 10 ml of sterile distilled water were put to get monospore suspension. The suspension obtained was filtered through Whatman paper (180 µm). The conidia were counted using a Malassez cell **(Belkacem-Hanfi et *al.*, 2014).**

Antifungal activity screening

Confrontation method is a qualitative test which aims to demonstrate the antifungal activity of the isolated bacteria. Strains were first inoculated in two parallel 2 cm stripes on MRS medium (without sodium acetate), and then incubated anaerobically at 30°C for 48h, then a 6 mm disc was taken from the peripheral zone of mold (5 days). The disc was placed in the center of the Petri dish and incubated at 30°C for 3 days under aerobic conditions in order to measure the diameter of fungi **(Laref et Guessas, 2013)**. The control was inoculated by the same method without LAB.

Antagonism assays

Overlay Method

To detect antifungal activity, overlay assay described by **Magnusson et *al.* (2003)** was used with modifications. Isolates were first inoculated in two stripes of 2 cm on MRS (15 ml) and incubated at 30°C for 48 h under anaerobic conditions. The colonies obtained were then covered with 10 ml of PDA medium (agar 0.9%) content 1ml of monospore suspension (10^5 conidia/ml). The assay without bacterial inoculum served as control. After 72h of incubation at 30°C, the inhibition zones were measured around bacteria streaks, according to the following criteria: (-): Absence of inhibition zone; (+): Inhibition zone: between 0.1 and 3% of the surface of the Petri dish; (++): Inhibition zone: between 3 and 8% of the surface of the Petri dish; (+++): Inhibition zone: greater than 8% of the surface of the Petri dish.

Wells method

This method is based on the principle that antimicrobials substances can diffuse into agar medium. Wells were made using punch on Petri dishes containing PDA medium supplemented with monospore suspension (10^5 conidia/ml). Fresh culture was centrifuged at 4600x g for 15 min and the supernatant was filtered using Millipore filters (Ø 0.22 µm) (Millex-GP, Bedford, OH, USA). A volume of 70 to 80µl of filtered supernatant were added to the wells. After 72 h of incubation at 30°C, the zones of inhibition were measured.

Antibacterial activity

To determine the supernatant activity spectrum of the retained LAB, Gram positive and Gram-negative indicator bacteria were tested, well diffusion method described by **Tagg and Mac Given (1971)** were performed. This indirect method allows the contact between LAB supernatant and the tested strains. LAB was cultivated in MRS broth and incubated for 18h at 30°C. After incubation, the culture was centrifuged at 4600x g for 15 min at 4°C and the supernatant was stored at 4°C. indicator strains were inoculated in Mueller-Hinton agar and wells were made with a punch and received 80µl of LAB supernatant. Plates were incubated for 24 h at 30°C.

Determination of the inhibitor's nature

Fresh cultures of LAB were centrifuged at 8450x g for 15 min at 4°C. The supernatant was neutralized with NaOH 5 mol/L to a pH =7 to eliminate the effect of organic acids. The proteolytic enzymes: trypsin (Sigma-Aldrich, Germany), pepsin (Sigma-Aldrich, Germany) and chymotrypsin (Sigma-Aldrich, Germany) were used to determine the nature of the inhibitory substances. The thermal sensitivity of substances was tested by heating at 80°C for 30 min. These substances were tested using the method of **Tagg and McGiven (1971).**

Supernatant concentration

The isolates which showed strong inhibitory activity were grown on MRS broth for 48 h at 30°C, then centrifuged at 8450x g for 15 min at 4°C. 50 ml of the supernatant obtained was added to an equal volume of absolute ethanol and then placed in a separating funnel for 2 days in order to separate the aqueous phase from the organic phase and the latter phase was collected. The solvent removal and the organic phase concentration were performed using rotary evaporator system (Büchi Rotavapor R-114, Büchi, Switzerland) at a temperature of 60°C. The dry residue was collected and redissolved in 2 ml of phosphate buffer (Na_2HPO_4 and NaH_2PO_4, 2 mM at pH 7).

RESULTS AND DISCUSSION

Isolation and selection of LAB

A total of 264 isolates were obtained. The initial phenotypic analysis of all isolates was distributed into 2 groups that were Gram (+), catalase (-) and non-spore forming. The first group represented rod forms 80 isolates (30.30%), the second group was cocci represented by 166 isolates (62.88%). A proportion of 19 isolates (7.2%) did not possess characters of LAB.
The cells association mode varied from one isolate to another. Isolates that had rod form, Gram $^{(+)}$ catalase $^{(-)}$ were chosen for the realization of this study.

Selection of antagonist strains by confrontation method

The confrontation method described above was used to determine the ability of the selected LAB isolates to inhibit mycelial growth. All of rods isolates were screened against three indicator molds: *Penicillium* sp., *A. fumigatus* and *A. niger*. This test revealed the presence of inhibitory activity for some isolates. A remarkable antagonistic activity was observed toward *Penicillium* sp. and varied against the other indicator strains. Among these, 14 isolates: Lma1, Lma2, Lma3, Lma4, Lma5, Lma6, Lma7, Lma8, Lma9, Lma10, Lma11, Lma12, Lma13 and Lma14 that process a considerable antifungal activity.

Figure 1 Moderate inhibition by *Lactobacillus rhamnosus* (Lma1) against *Aspergillus niger* using the confrontation method.

Results obtained by the confrontation method illustrated the ability of *Lactobacillus* ssp. isolates to inhibit *A. niger, A. fumigatus* and *Penicillium* sp.

The fourteen selected isolates showed an inhibitory effect on the three fungal indicators. These lactobacilli were selected according to their antifungal abilities in tests carried out by the confrontation method. The isolates could affect the mycelial growth with various degrees of inhibition for each isolate and also for each indicator strain. In addition, we recorded that 74% of the isolates showed a high inhibition rate to *Penicillium.* It has been noted that the strain Lma3 showed antifungal efficacity by strong inhibition against the three molds. These results are similar to the results cited by **Fhoula et *al.* (2013)** for LAB isolated from rhizosphere of olive trees and desert truffles on *Botrytis cinerea*, *Penicillium expansum*, *Verticillium dahliae*, and *Aspergillus niger*. The production of antifungal compounds depends on the selected strain, the growth conditions, and the interactions between fungal and bacterial metabolites **(Laref et *al.*, 2013).**

Table 1 Carbohydrates fermentation of *Lactobacillus* isolates obtained by API 50 CHL analyses

strains	Lma5	Lma11	Lma3	Lma1	Lma10	Lma7	Lma9	Lma2	Lma4	Lma6	Lma8	Lma13	Lma12	Lma14
Tests														
W	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GLY	-	-	-	-	+	-	-	-	-	-	-	-	-	-
ERY	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DARA	-	-	-	+	-	-	-	-	-	-	-	-	-	-
LARA	-	-	+	-	-	-	-	+	+	+	+	+	+	+
RIB	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DXYL	-	-	-	-	-	-	-	-	+	-	+	-	-	-
LXYL	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADO	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDX	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FRU	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MNE	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SBE	-	+	-	+	+	+	+	-	-	-	-	-	-	-
RHA	-	-	-	+	-	-	-	-	+	-	-	-	-	-
DUL	-	-	-	-	-	-	-	-	-	-	-	-	-	-
INO	-	+	-	-	-	-	-	-	-	-	-	-	-	-
MAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SOR	+	+	-	+	+	+	+	+	+	+	+	+	+	+
MDM	-	-	-	-	-	-	-	-	-	+	-	+	+	-
MDG	-	-	-	+	-	-	-	-	-	-	-	-	-	-
NAG	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AMY	+	+	+	+	+	+	-	+	+	+	+	+	+	+
ARB	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESC	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CEL	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LAC	+	+	-	+	+	+	+	+	+	+	+	+	+	+
MEL	-	-	+	-	-	-	-	+	+	+	+	+	+	+
SAC	+	+	+	-	+	+	+	+	+	+	+	+	+	+
TRE	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INU	-	+	-	-	-	-	-	-	-	-	-	-	-	-
MLZ	+	+	-	+	+	+	+	+	+	+	+	+	+	+
RAF	-	-	-	-	-	-	-	+	-	+	+	+	+	+
AMD	-	+	-	-	-	-	-	-	-	-	-	-	-	-
GLYG	-	-	-	-	-	-	-	-	-	-	+	-	-	-
XLT	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GEN	-	+	+	+	+	+	+	+	+	+	+	+	+	+
TUR	+	+	-	+	-	+	-	+	+	+	+	+	+	+
LYX	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TAG	+	+	-	+	+	+	+	-	+	-	+	-	-	-
DFUC	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LFUC	-	-	-	+	+	-	-	-	-	-	-	-	-	-
DARL	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LARL	-	+	-	-	-	-	-	-	-	-	-	-	-	-
GNT	+	+	+	-	+	+	+	+	-	+	+	+	+	+
2KG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5KG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Identified as	***Lactobacillus paracasei* subsp. *paracasei***	***Lactobacillus paracasei* subsp. *paracasei***	***Lactobacillus brevis***	***Lactobacillus rhamnosus***	***Lactobacillus paracasei* subsp. *paracasei***	***Lactobacillus paracasei* subsp. *paracasei***	***Lactobacillus paracasei* subsp. *paracasei***	***Lactobacillus plantarum***	***Lactobacillus pentosus***	***Lactobacillus plantarum***	***Lactobacillus plantarum***	***Lactobacillus plantarum***	***Lactobacillus plantarum***	***Lactobacillus plantarum***
ID %	**99,9**	**99,9**	**94.4**	**99.9**	**98.2**	**99.5**	**98.2**	**99.4**	**77.7**	**99.9**	**95.7**	**99.9**	**99.9**	**99.4**

Legend: W: Witness, GLY: Glycérol, ERY: Erythritol, DARA: D- Arabinose, LARA: L- Arabinose, RIB: D- Ribose, DXYL: D- Xylose, LXYL: L- Xylose, ADO: D- Adonitol, MDX: Méthyl-D-Xylopyranoside, GAL: D- Galactose GLU: D- Glucose, FRU: D- Fructose, MNE: D- Mannose, SBE: L- Sorbose, RHA: L- Rhamnose, DUL: Dulcitol, INO: Inositol, MAN: D-Mannitol, SOR: D- Sorbitol, MDM: Méthyl-□D-Mannopyranoside, MDG: Méthyl-□D-Glucopyranoside, NAG:N AcétylGlucosamine, AMY: Amygdaline, ARB:Arbutine, ESC:Esculin iron citrate SAL: Salicine, CEL : D- Celiobiose, MAL: D- Maltose, LAC :D- Lactose, MEL: D- Melibiose, SAC: D-Saccharose, TRE: D- Trehalose, INU : Inuline, MLZ : D- Mélézitose, RAF : D- Raffinose, AMD : Amidon, GLYG : Glycogène, XLT : Xylitol, GEN: Gentiobiose, TUR: D-Turanose, LYX: D- Lyxose, TAG: D- Tagatose, DFUC: D- Frucose, LFUC: L-Frucose, DARL: D- Arabitol, LARL: L-Arabitol, GNT: Potassium Gluconate, 2KG: Potassium 2-cétogluconate, 5KG: Potassium 5-cétogluconate

Isolates identification

Genus level identification

Isolates were classified as belonging to the genus *Lactobacillus*. All isolates were able to grow at 15°C, 2%, and 4% NaCl. They were grouped according to CO_2 production from glucose, gluconate and NH_3 production from arginine. Gas production (CO_2) from glucose, was negative for the 14 isolates. Whereas, in gas production from gluconate all isolates produce CO_2. These results show that all isolates are optional heterofermentative. The growth at 45°C was observed in 9 isolates. Only one isolate Lma11 that can hydrolyze arginine.

After performed morphological characters and growth at different temperatures (15°C, 45°C), fermentation type (from glucose and gluconate), arginine hydrolysis and ribose fermentation (14 isolates ferment ribose), following the recommendations of **Carr et *al.* (2002), Axelsson (2004)** and **Hammes and Hertel (2006)**, we can conclude that the fourteen isolates belonging to the genus *Lactobacillus*. All isolates are classified in Group II (Streptobacteria) and are optional heterofermentative.

Table 2 Results of the fermentation type (glucose/Gluconate), arginine dehydrogenase activity, ribose fermentation, growth in NaCl and growth temperatures.

Strains	CO_2 from Glucose	CO_2 from gluconate	ribose	2% NaCl	4% NaCl	ADH	15°C	45°C
Lma1	-	+	+	+	+	-	+	-
Lma2	-	+	+	+	+	+	+	+
Lma3	-	+	+	+	+	-	+	+
Lma4	-	+	+	+	+	-	+	-
Lma5	-	+	+	+	+	-	+	+
Lma6	-	+	+	+	+	-	+	+
Lma7	-	+	+	+	+	-	+	+
Lma8	-	+	+	+	+	-	+	-
Lma9	-	+	+	+	+	-	+	-
Lma10	-	+	+	+	+	-	+	+
Lma11	-	+	+	+	+	-	+	+
Lma12	-	+	+	+	+	-	+	+
Lma13	-	+	+	+	+	-	+	+
Lma14	-	+	+	+	+	-	+	-

Species level identification

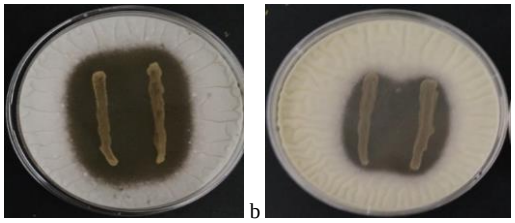
The results of carbohydrates fermentation on the API gallery 50 CHL, were used to identify species. Based on these results, and following instructions of **Carr et al. (2002), Axelsson (2004) and Hammes and Hertel (2006)**, we classified strains: Lma14, Lma12, Lma13, Lma6, Lma8, Lma2 belong to the species *Lb. plantarum*. The Lma5, Lma11, Lma10, Lma7, Lma9 strains belong to the species *Lb. paracasei* subsp. *paracasei*. The strain Lma3 belongs to the species *Lactobacillus brevis*. The strains Lma1 and Lma4 were assigned to the species *Lb. rhamnosus* and *Lb. pentosus* respectively. Study realized by **Khedid et *al.* (2009)** on camel's milk microflora shows that *Lb. plantarum, Lb. paracasei, Lb. brevis* are the dominant species. Our study reveals another species *Lb. rhamnosus* and *Lb. pentosus* which have an antifungal activity.

Overlay assay method

The fourteen (14) Lactobacillus showed an inhibitory activity against the 03 fungi indicator. Overlay assay test revealed also a varied degree of activity against conidia. This remarkable activity differs from one LAB isolate to another and also from a fungal strain to another. All selected isolates (92%) showed a moderate (++) to strong (+++) antifungal effect on the conidia of *A. fumigatus* and *Penicillium* sp. *A. niger* is the most resistant with low inhibitory activity (+) to moderate (++). The lower antifungal activity against the 3 fungi was recorded for *Lb. plantarum* (Lma8), this LAB could not inhibit spore germination of *A. niger*. However, the strain *Lb. rhamnosus* (Lma1) is the most effective antagonist strain against *Penicillium* sp., *A. fumigatus* and *A. niger* (Figure 2).

Seven (07) LAB isolates showed weak antifungal activity (+) against *A. niger*, five (05) isolates showed a moderate activity (++). Our results showed that LAB isolates have a weak to moderate antifungal activity against *A. niger*. For *A. fumigatus*, 21% of the LAB isolates showed a strong antifungal activity (+++), the rest of the isolates showed moderate activity, with an isolate that showed low activity against this strain. The strong antifungal activity observed (+++) was against *Penicillium* sp. with 78.57%.

Works of **Ström et *al*. (2002), Magnusson et *al.* (2003), Rouse et *al.* (2008), Laref et *al*. (2013)** and **Belkacem-Hanfi et *al*. (2014)** confirmed the antifungal activity of lactobacilli on conidia germination of *Aspergillus* ssp. and *Penicillium* sp.



a b

Figure 2 Antifungal activity of *Lactobacillus rhamnosus* (Lma1) using the overlay technique. (a) *Penicillium* sp. (b) *A. fumigatus*.

The retained *Lactobacillus* spp. isolates were able to inhibit the mycelia growth and the conidia germination, this later were more affected than the mycelia. This result is similar to those cited by **Laref and Guessas (2013)**.

Antifungal activity of *Lb. plantarum* are reported against *Colletotrichum gloeosporioides* **(Elmabrok et *al*., 2013)**, *Aspergillus flavus* and *Penicillium expansum* **(Cortés-Zavaleta et *al*., 2014)** and *Penicillium roqueforti* **(Yan et *al*., 2017)**. The antifungal activity is also described for *Lb. paracasei* toward *Aspergillus flavus, Aspergillus niger* and *Penicillium glaucum* **(Miao et *al*., 2014)**, *Penicillium sp.* **(Aunsbjerg et *al*., 2015)** and *Aspergillus* **(Inglin et *al*., 2015)**. Studies carried out by **Coman et *al.* (2014), Delavenne et *al.* (2015)** and **Inglin et *al.* (2015)** demonstrate that *Lb. rhamnosus* possess an antifungal activity against *Aspergillus, Penicillium, Candida, Rhodotorula, Kluyveromyce, Yarrowia, Botrytis and Colletotrichum.* Approved antifungal activity has been noted for *Lb. pentosus* toward *Candida albicans* **(Crowley et al., 2013)**, *Geotrichum, Alternaria, Fusarium, Aspergillus and Penicillium* **(Lipińska et *al*., 2017)**. The antifungal action of *Lactobacillus brevis* is cited by the authors **Tropcheva et *al*. (2014), Arasu et *al*. (2015)** and **Axel et *al*. (2016)**.

No antifungal activity was recorded by the free-cell supernatant for all isolates, the same results were obtained by **Schwenninger and Meile (2004)** and **Laref and Guessas (2013).**

Table 3 Antifungal activity tested by the overlay method

Strains	Isolates code	*A. niger*	*A. fumigatus*	*Penicillium* sp.
Lactobacillus rhamnosus	Lma1	++	+++	+++
Lactobacillus plantarum	Lma2	ND	++	+++
Lactobacillus brevis	Lma3	++	++	+++
Lactobacillus pentosus	Lma4	+	++	+++
Lactobacillus paracasei subsp. paracasei	Lma5	++	++	++
Lactobacillus plantarum	Lma6	+	+++	+++
Lactobacillus paracasei subsp. paracasei	Lma7	++	++	+++
Lactobacillus plantarum	Lma8	-	+	++
Lactobacillus paracasei subsp. paracasei	Lma9	+	++	+++
Lactobacillus paracasei subsp. paracasei	Lma10	++	++	+++
Lactobacillus paracasei subsp. paracasei	Lma11	+	++	+++
Lactobacillus plantarum	Lma12	+	+++	+++
Lactobacillus plantarum	Lma13	+	++	++
Lactobacillus plantarum	Lma14	+	++	+++

Legend: (-): Absence of inhibition zone; (+): Inhibition zone between 0.1 and 3% of the surface of the Petri dish (low); (++): Inhibition area between 3 and 8% of the surface of the Petri dish (moderate); (+++): Inhibition area greater than 8% of the surface of the Petri dish. (Strong); (ND): not determined

Antibacterial activity

In addition to the antifungal activity, LAB isolates revealed also a high antibacterial activity spectrum, the results showed the effect of unbuffered supernatant on the growth of *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *L. ivanovii* ATCC 19119 and *P. aeruginosa*. The results showed that 14 strains (100%) had an inhibition against *P. aeruginosa, E. coli and S. aureus*, 05 of these isolates (36%) showed a strong inhibitory activity. However, (85.71%) affect *L. ivanovii*. This activity has been recorded for *Lb. brevis* (Lma3) isolate, *Lb. rhamnosus* (Lma1), *Lb. plantarum* (Lma2), *Lb. plantarum* (Lma8) and *Lb. paracasei* subsp. *paracasei* (Lma9). Six isolates (43%) showed strong inhibitory activity against *E. coli*. 06 strains affect *L. ivanovii* ATCC 19119 with moderate activity. Six (06) lactobacilli also showed a strong inhibitory activity against *P. aeruginosa* (diameter varied between 17 to 22 mm). Lactobacilli's Isolates showed a wide range of antibacterial activity on both of Gram (-) and Gram positive (+) indicator: *E. coli* ATCC 25922, *P. aeruginosa, L. ivanovii* ATCC 19119 and *S. aureus* ATCC 25923. Inhibition of *E. coli* by *Lactobacillus* strains has already been described **(Todorov et *al.*, 2004; Karthikeyan and Santosh, 2009)**. **Todorov et *al.* in 2004** and **Karthikeyan & Santosh in 2009** had recorded the inhibition of the strain of *P. aeruginosa* by *Lactobacillus* ssp. The anti-Listerial activity of *Lactobacillus* has been described by **Ouwehand and Vesterlund (2004).** In **2008 Mami and collaborators** mention that *Lactobacillus* strains isolated from raw goat milk inhibit *S. aureus* strains.

Table 4 Antibacterial inhibition diameter (mm) of LAB strains on bacterial indicator

Lactic acid bacteria Strains test	Lma1	Lma2	Lma3	Lma4	Lma5	Lma6	Lma7	Lma8	Lma9	Lma10	Lma11	Lma12	Lma13	Lma14
S. aureus	20±0.00	17±1.73	20±1.73	14±1.00	14±2.64	13±2.64	13±1.00	16±1.00	15±1.73	21±1.00	17±1.00	13±1.00	16±1.00	18±0.00
E. coli	23±0.00	19±3.60	20±2.64	15±1.00	15±1.00	18±1.00	14±1.00	17±1.00	16±0.00	21±1.00	14±1.00	18±0.00	12±2.64	19±1.00
P. aeruginosa	21±0.00	18±0.00	19±0.00	16±1.00	16±1.00	13±1.00	15±0.00	18±1.00	22±1.00	15±0.00	12±0.00	13±1.00	14±1.00	16±1.73
L. ivanovii	12±1.00	13±0.00	14±1.00	07±0.00	17±0.00	13±1.00	17±0.00	15±1.00	13±0.00	16±0.00	08±0.00	13±1.00	17±1.00	14±0.00

Table 5 Inhibition diameter (mm) of the neutralized LAB free-cell supernatant against *P. aeruginosa.*

	Lma6	Lma4	Lma13	Lma8	Lma14	Lma5	Lma7	Lma2	Lma11	Lma3	Lma1	Lma12	Lma10	Lma9
P. aeruginosa	17±1.00	14±1.73	12±0.00	20±1.00	19±1.00	18±1.00	26±3.60	27±1.73	20±1.00	21±0.00	19±1.00	24±1.00	18±1.00	23±0.00

The concentrated filtrate

The free-cell concentrated supernatant of *Lb. brevis* (Lma3) was able to inhibit both of all fungal and bacterial strains**.** *Penicillium* sp. is the most sensitive mold with 18 mm zone of inhibition, followed by A. *fumigatus* (16 mm). *A. niger* showed resistance to concentrated filtrate as the inhibition zone did not exceed 12 mm. Concentered free-cell supernatant affect also *E. coli*, *L. ivanovii*, *P. aeruginosa* and *S. aureus* with diameters of inhibitions between 16 to 22 mm. It has been noted that *S. aureus was* the most sensitive against the free-cell concentrated supernatant. **Wang and collaborators in 2012** showed the effect of the concentrate filtrate of *Lb. plantarum* against *P. citrinum* and *P. digitatum.* **Saraniya and Jeevaratnam (2014)** showed that *S. aureus* MTCC 737 was inhibited by the concentrate supernatant (10 times) of *Lb. pentosus*. The antimicrobial effect of the concentrate free-cell supernatant *Lb. plantarum* on *P. aeruginosa* and *E. coli* has been also reported by **Smaoui et *al.* (2010).**

Determination of inhibitor nature

Neutralizing pH, treatment with proteolytic enzymes and heating of free-cell supernatant revealed that no inhibitory effect on *A. niger*, *A. fumigatus*, *Penicillium* sp., *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *L. ivanovii* ATCC 19119. However, this inhibitory effect persisted on the *P. aeruginosa* strain. Laref et al. (2013) motioned this persistence of inhibitory effect on this strain.

Neutralization of free-cell supernatant can eliminate the activity of antifungal compounds. **Magnusson and Schnurer (2001)** described that the antifungal substance is activated in low pH, this peptide was stable at pH values between 3.0 and 4.5 but decreased between 4.5 and 6.0., In addition, the study of **Rouse et van Sinderen (2008)** had confirmed that treatment of the concentrated supernatant of *Lb. plantarum* with proteinase K suppress the antifungal activity. **Miescher Schwenninger et *al.* (2005)** reported that heating for 10 min at 100°C of the supernatant of *Lb. paracasei* subsp. *paracasei* isolates don't affect the antifungal activity. The antifungal activity of lactobacilli is less well characterized, but organic acids, as still uncharacterized proteinaceous compounds, and cyclic dipeptides can inhibit the growth of some fungi **(Rouse et van Sinderen, 2008)**. Organic acids produced by *Lactobacillus* ssp., such as 3-hydroxy fatty acids and phenyllactic acid, have been recently described for their inhibitory effect on the growth of some fungal **(Lee et *al.*, 2016; Russo et *al.*, 2017; Lipińska et *al.*, 2017; Dinev et *al.*, 2017)**. *Lb. rhamnosus* been reported to inhibit the growth of many spoilage and toxigenic fungi including species in the genera *Aspergillus* and *Penicillium* **(Plockova et *al.*, 2001)**. **Iglesias et *al.* (2018)** cited that the antifungal activity of *Lb. rhamnosus* GG can be related to emissions of many volatiles compounds. Recently, number of antifungal metabolites: Cyclo-(Leu-Pro), 2,6-diphenyl –piperidine, and 5,10-diethoxy-2,3,7, 8-tetrahydro-1H, 6H-dipyrrolo[1,2-a;10,20-d]pyrazine, Phenyllactic acid, 3-Propanoic acid, Sinapic acid, 2-Deoxycytidine, Cyclo (L-His-L Pro), Cyclo (L-Tyr-L Pro), Phenylpyruvic acid, cis-Caftaric acid, Protocatechuicacidhexoside, Caffeic acid derivative, Hydroxycinnamic acid derivative, Quercetinpentoside, Quinic acid derivative, Caffeoylhexose-deoxyhexoside, 3,5-Di-O-caffeoylquinic acid are isolated from *Lactobacillus* species **(Li et *al.*, 2012; Yépez et *al.*, 2017).**

Table 6 Inhibition Diameter (mm) of concentered free-cell supernatant of *Lactobacillus brevis* (Lma3) compared with the ethanol against fungal and bacterial indicator

	P. aeruginosa	*S. aureus*	*E. coli*	*L. ivanovii*	*A. niger*	*A. fumigatus*	*Penicillium sp*
Concentered supernatant	19±1.00	22±1.00	16±1.00	18±1.00	12±1.00	16±1.00	18±1.00
Ethanol	13±1.00	14±1.00	13±1.00	15±1.00	11±1.00	12±1.00	13±1.00
Difference	6	8	3	3	1	4	5

CONCLUSION

This paper reported the isolation and identification of LAB from Algerian camel's milk. The results showed a high rate of antifungal activity, indicating that camel's milk may be source for the selection of new LAB with important technological potential, which are useful for the biocontrol of food, plant, fungi and pathogenic bacteria. These antifungal strains LAB have potential characters to be used in food preservation to inhibit conidia germination and mycelia growth of spoilage fungi. Further investigations to elucidate the nature of inhibiting compounds should be considered.

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