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ISOLATION OF LACTIC ACID BACTERIA FROM TRADITIONAL MOROCCAN PRODUCTS AND EVALUATION OF THEIR ANTIFUNGAL ACTIVITY ON GROWTH AND OCHRATOXIN A PRODUCTION BY *ASPERGILLUS CARBONARIUS* AND *A. NIGER*

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ABSTRACT

Different agricultural products, including grapes, are susceptible to contamination and damage by several microorganisms, one being fungi and their mycotoxins such as ochratoxin A. These alterations are a source of food waste and lead to significant economic losses. Thus, new alternatives have been sought in parallel with existing methods. In this work, after isolation, identification by 16S rDNA sequencing and differentiation by Pulsed-Field Gel Electrophoresis (PFGE) of four lactic acid bacteria from different traditional Moroccan food products, antifungal activity was studied against *Aspergillus carbonarius* and *A. niger* isolated from Moroccan grapes. Their effect on ochratoxin A production was also assessed. A strain of *Lactococcus lactis* ssp. *lactis* (F) and three strains of *Leuconostoc mesenteroides* ssp. *mesenteroides* (I, C, and D) selected from 16 isolated strains were tested. Their antifungal activities against *Aspergillus* spp. were quite significant, with growth inhibition rates ranging from 28.69% to 53.31%. Likewise, ochratoxin A production by *Aspergillus* strains was significantly reduced. Indeed, in the presence of the *Lactoccocus lactis ssp. lactis* (F) strain, this reduction was about 63.90% and 65.12% for *A. niger* and *A. carbonarius,* respectively; however, an 89.38% reduction for *A. niger* and 19.35 % *A. carbonarius* was achieved for the *Leuconostoc mesenteroides* ssp. *mesenteroides* (I) strain. These results revealed strain-dependent antifungal activity. Our study indicates that the use of these lactic acid bacteria can be applied as a biocontrol of fungal growth and mycotoxins production.

Keywords: *Aspergillus* sp., growth inhibition, *Lactoccocus lactis* ssp. *lactis*, *Leuconostoc mesenteroides* ssp. *mesenteroides,* OTA reduction

INTRODUCTION

Deterioration of food by filamentous fungi is a major preoccupation for farmers and the food industry due to their capacity to grow under harsh environmental conditions; they limit the shelf life of food products and cause a 5-10% loss in world crops **(Pitt & Hocking, 2009)**. Molds produce compounds that have a negative impact on organoleptic properties, such as the molecules responsible for flavor defects, alteration of texture, or discoloration of food products **(Muhialdin *et al.,* 2013)**. In addition, some spoilage fungal species such as *Aspergillus* sp. can produce mycotoxins, including ochratoxin A (OTA). OTA is among the most studied mycotoxins because of its damaging effects on human and animal health, inducing nephrotoxic, carcinogenic possible effects, teratogenic, immunotoxic, and neurotoxic reactions **(IARC, 1993; Pfohl-Leszkowicz & Manderville, 2007)**. In grapes and their derivatives, the most important species of *Aspergillus* sp. OTA producers are *Aspergillus niger aggregate* and *Aspergillus carbonarius* **(Cabanes & Bragulat, 2018; Nielsen *et al.,* 2009; Selouane *et al.,* 2009; Leong, 2005)**. Several chemical and physical preservation methods are used to inhibit mold growth and mycotoxins production in food products **(Farkas, 2001; Legan, 1993)**; however, the overuse of these chemicals is increasingly discouraged for economic reasons and because of growing concerns with environmental and food safety issues **(Wagacha & Muthomi, 2008)**. As such, research for new methods to reduce the risk of fungal contamination is necessary, and the potential exploitation of the antifungal properties of lactic acid bacteria (LAB) for food bio-preservation has attracted the attention of several researchers **(Muhialdin *et al.,* 2013)**. LAB can be found naturally in different foodborne products **(Carr *et al.,* 2002; Gajbhiye & Kapadnis, 2016)**. They have been widely used in traditional fermentation processes since they are Generally Recognized As Safe (GRAS) and have important technological properties, such as inhibiting the growth of fungi by producing antifungal compounds **(Gajbhiye and Kapadnis, 2016)**. In this work, the antifungal activity of lactic acid bacteria was evaluated. Lactic acid bacteria were isolated from different Moroccan biotopes, and the screening of strains with antifungal activity against *A. carbonarius* and *A. niger* was investigated. The effect of the selected isolate bacteria on OTA production by the *Aspergillus* sp. was also studied.

MATERIAL AND METHODS

Fungal strains and growth conditions

Two *Aspergillus* strains, *Aspergillus niger* (MUCL 49226) from raisins and *Aspergillus carbonarius* (MUCL 49345) from fresh Moroccan grapes, were isolated and identified by **Selouane *et al.* (2009)**, then deposited at BCCM / MUCL (Mycothèque de l'Université catholique de Louvain, Louvain-la-Neuve, Belgium) and used in this work. The spore inocula were prepared from fungi culture on Czapek Yeast Autolysate Agar (CYA) medium (Sucrose, 30g ; yeast extract, 5g ; $NaNO_3$, 2g ; KCl, 0.5g ; $MgSO_4$, 0.5g ; $FeSO_4$, 0.07g ; K_2HPO_4, 1g ; $ZnSO_4.7H_2O$, 10 mg, $CuSO_4$, 5 mg; Agar, 15g ; distilled water, 1 liter) at 25 °C for 7 days. Spore suspensions were prepared in sterile distilled water containing Tween 80 (0.001%) at 5 °C. Malassez cell was used to determine the final spore concentrations (~10^5 spores/mL) according to **Wiktor (2008)**.

Isolation of lactic acid bacteria

The strains of lactic bacteria were isolated from different Moroccan biotopes and food products such as milk and its traditional derivatives (Jben, Lben, and Smen) as well as brine from fermented olives and fermented capers. Serial decimal dilutions were made in a physiological water solution, then 0.1 mL from dilutions

of 10^{-4}, 10^{-5}, and 10^{-6} were inoculated on the surface of Petri plates containing MRS **(De Man, Rogosa and Sharpe, 1960)** agar medium. The plates were incubated at 35 °C for 24 to 48 h. The colonies were selected, purified, and preliminarily identified.

Screening of the antifungal activity of lactic acid bacteria

The antifungal activity of lactic acid bacteria was tested using the double layer method described by **Cheong *et al.* (2014)** with some modifications. The bacteria were inoculated on two streaks on Petri plates. Two culture media, MRS (Tryptic digest of casein, 10 g ; beef extract, 8 g ; yeast extract, 4 g ; glucose, 20 g ; Tween 80, 1 g ; di-potassium hydrogen orthophosphate, 2 g ; magnesium sulphate $7H_20$, 0.2 g ; manganese (II) sulphate $4H_20$, 0.05 g ; ammonium citrate, 2 g ; sodium acetate $3H_20$, 5 g ; agar, 15g ; distilled water, 1 litre) and M17 (Peptone, 5.0 g ; soya peptone, 5.0 g ; yeast extract, 2.5 g ; beef extract, 5.0 g ; lactose, 5.0 g ; sodium glycerophosphate, 19.0 g ; magnesium sulfate, 0.25 g ; ascorbic acid, 0.5 g ; agar, 15 g ; distilled water, 1 liter; **Terzaghi & sandine, 1975**) were used to compare the influence of culture media on antifungal activity. The plates were subsequently incubated at different temperatures (25, 30 and 37 °C) and for different times (18, 24 and 48 h). After incubation, the Petri plates were covered by 10 mL of CYA containing 10^5 spores/mL and incubated at 25 °C for 72 h. Control plates of MRS agar medium were used to inoculate the two strains of *Aspergillus* sp. to eliminate the effect of sodium acetate, as an antifungal agent.
The following scale was used for the selection of lactic acid bacteria strains with potential antifungal activity: (-), no inhibition (the bacteria were totally covered by the fungal strain); (+), weak inhibition (the bacteria were not covered by the fungi); (++), inhibition (formation of a small zones inhibition around the bacterial culture); and (+++), strong inhibition (a large zones of inhibition has been observed around the bacterial culture). The antifungal activity tests were conducted in triplicate.

Identification of selected lactic acid bacteria by 16S rDNA sequencing

The selected lactic acid isolates were inoculated in MRS broth medium and then incubated at 30 °C for 24 h. For each isolate studied, the DNA was extracted using a Qiagen Blood and Tissue kit (Qiagen, Germany). The concentration of extracted DNA was then measured using Nanodrop® (Thermo Fisher Scientific, USA).
First, the extracted DNA was amplified by polymerase chain reaction (PCR) using universal primers W001 and W002 **(Godon *et al.*, 1997)**. Then, it was sequenced according to the Sanger method **(Sanger & Coulson, 1975)**. The sequencing results were analyzed using Invitrogen-Vector NTI software, and the sequence obtained was compared with the sequences in the NCBI (Nucleotide Blast) database (https://www.ncbi.nlm.nih.gov/). Finally, the strains were subjected to species-specific PCR in order to differentiate the subspecies.

Differentiation of lactic acid bacteria strains selected by Pulsed-Field Gel Electrophoresis (PFGE)

Clonal differentiation of the selected strains by PFGE was conducted. This technique is largely used for the analysis of genomic diversity of lactic acid bacteria **(Adesulu-Dahunsi *et al.*, 2017)**. For this, the strains were cultivated in MRS medium at 30 °C for 24 h and analyzed. After migration, profiles were analyzed using Bionumerics software (AppliedMath, Belgium). The gel was first normalized using a molecular weight marker. The results were compiled in the form of a dendogram for comparing the samples with each other by considering the number of bands and their position. Two samples with the same number of bands at the same positions were considered identical (duplicates of the same strain).

Determination of the antifungal activity of bacterial supernatants

The isolates of lactic acid bacteria were inoculated in 10 mL of MRS broth medium for 18 h at 30 °C. An aliquot (200 µL) of each culture was then inoculated into MRS broth medium (20 mL) and incubated at 30 °C for 48 h. After centrifugation (7200 × g, 10 min) (Sigma 1-14, Sigma Laborzentrifugen GmbH, Germany), the supernatants obtained were sterilized by filtration (Millipore 0.45 µm) and immediately tested for their antifungal activities according to **Cortés-Zavaleta *et al.* (2014)** with some modifications. The bacterial supernatant was mixed with CYA agar medium at 45 °C to reach a final concentration of 5% (v/v) and poured into Petri plates (15 mL per plate). Next, 10 µL of the spore suspension was placed in the center of the plates. Control plates containing only CYA agar medium mixed with MRS broth medium in the same proportions (5%, v/v) were used. During incubation at 25 °C, the diameters of the mold growth were measured daily for 7 days, and the percentage of growth inhibition (I) % was calculated according to the following formula:

$$I = 100 \times (A_C - A_T) / (A_C),$$

Where A_C and A_T are the diameters of mycelial growth in treated and control plates, respectively. All analyses were performed in triplicate.

Ochratoxin A extraction and analysis by High-performance liquid chromatography with fluorescence detection (HPLC-FLD)

Ochratoxin A (OTA) was extracted using the method described by **Bragulat *et al.* (2001)**. Three agar plugs (diameter = 7 mm) were taken from the inner, central, and outer areas of each colony. After the plugs were weighed and distributed in flasks, 1 mL of methanol (99.9 %) was added, then the mixture was shaken for 5 s and incubated at 25 °C for 60 min. The extracts were centrifuged (HERMLE Z 230 MA, Labnet International, USA) three times for 10 min at 13,000 rpm. The supernatant was filtered on a hydrophilic PVDF filter (0.22 µm), then analyzed by HPLC (Agilent Technologies, USA) with fluorescence detection (FLD) (excitation at 333 nm, emission at 460 nm; calibration with OTA standard (Sigma Aldrich, Steinheim, Germany). The metabolites were separated on a C18 reverse phase column (Zorbax SB, particle size 4.6 × 250 mm × 5 µm).
OTA was analyzed in isocratic mode. The mobile phase (acetonitrile-water-acetic acid; 99: 99: 2, v/v/v) was pumped at 0.7 mL/min with an injection volume of 20 µL. OTA was detected at around 11 min. By comparing the retention time of the peaks of the extract with the OTA standard and with co-injection, the final concentration (expressed in ng/g of CYA) was determined based on the calibration curve established for each series. All analyses were performed in triplicate.

RESULTS

Isolation of lactic acid bacteria

As previously described, lactic acid bacteria were isolated from various natural products of different Moroccan origin, including raw milk (cow, sheep, goats and camel), Smen, Jben, Lben, as well as brine of fermented olives and capers. Among 66 isolates, only 16 isolates were considered lactic acid bacteria since they were Gram-positive, catalase and oxidase negative, and immobile (Table 1). These isolates were tested for antifungal activity against *A. carbonarius* and *A. niger.*

Table 1 Origin and source of lactic acid bacterial isolates used for the study of antifungal activity

Isolates code	Origin (city)	Source of isolation	Gram	Catalase
A	Fez	Cow milk	Positive	Negative
B		Cow milk	Positive	Negative
C		Lben (Traditional fermented cow's milk)	Positive	Negative
D		Lben (Traditional fermented cow's milk)	Positive	Negative
E		Cow milk	Positive	Negative
F		Fermented Capers	Positive	Negative
G		Fermented Capers	Positive	Negative
H		Cow milk	Positive	Negative
I		Lben (Traditional fermented cow's milk)	Positive	Negative
Lb		Sheep milk	Positive	Negative
Ca	Taounate	Fermented Capers	Positive	Negative
Lv	Chefchaoun	Goat's milk	Positive	Negative
Se	Tangier	Smen (Traditional butter)	Positive	Negative
EO	Rabat	Fermented olive water	Positive	Negative
Ld	Dakhla	Camel milk	Positive	Negative
Lc	Errachidia	Camel milk	Positive	Negative

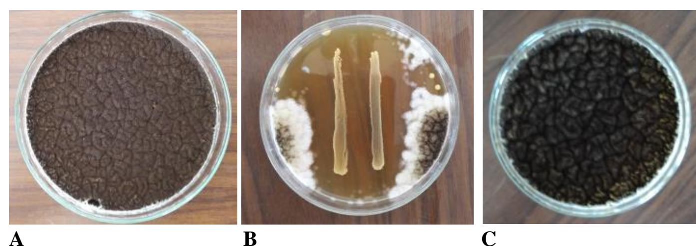
Screening of the antifungal activity of lactic acid bacteria and molecular identification

According to the results of the 16 isolates studied, only four, named (F), (I), (C), and (D), were found to inhibit the growth of the two *Aspergillus* sp. tested. They were incubated at temperature of 30 °C for 48 h (Table 2, Figure 1); however, no inhibition was observed for the 16 isolates incubated at 25 °C and 37 °C for incubation periods of 18 h and 24 h, respectively. In addition, the MRS agar medium appeared to promote the production of antifungal substances by the isolates (F), (I), (C), and (D) because the zones of inhibition that appeared on the MRS agar medium were larger than those on the M17 agar medium (Figure 2). Isolates (F), (I), (C), and (D) were retained for the following studies.

Table 2 Antifungal activity of lactic acid bacteria selected isolates using double layer method after incubation at 30 °C for 48 h

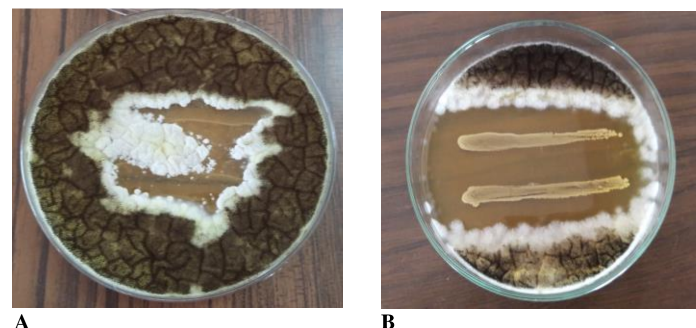
Tested strains	F	E	G	H	A	C	B	I	D	Lb	Ld	Lv	Se	EO	ca	Lc
Aspergillus carbonarius (MUCL 49345)	+++	-	-	-	-	++	-	+++	++	-	-	-	-	-	-	-
Aspergillus niger (MUCL 49226)	+++	-	-	-	-	++	-	+++	++	-	-	-	-	-	-	-

(-): no antifungal activity, (++): moderate activity, (+++): high antifungal activity



A B C

Figure 1 Growth of *Aspergillus niger* on CYA agar medium (control, **A**), inhibitory zone of *A. niger* due to the growth of isolate (F) after incubation at 30 °C/48 h **(B)** and growth of *A. niger* on MRS agar medium (control, **C**)



A B

Figure 2 Comparison of the inhibition zones observed due to the growth of isolate (I) on M17 **(A)** and MRS **(B)** culture media

Molecular identification and differentiation of lactic isolates selected by PFGE (Pulsed-Field Gel Electrophoresis)

The four retained isolates were molecularly identified as *Leuconostoc mesenteroides* ssp. *mesenteroides* for (I), (C), and (D) and as *Lactococcus lactis* ssp. *lactis* for (F). After differentiation by PFGE of the three species of *Leuconostoc* (I, C and D) and after analysis of the dendrogram (Figure 3), it appeared that the three isolates are different but genetically similar with high percentages of similarity; (C) and (D) displayed 93.8% similarity, (I) and (D) displayed 97% similarity, and (I) and (C) displayed 95.3% similarity.

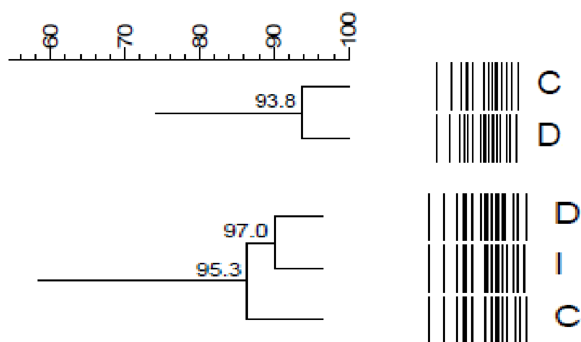


Figure 3 Dendrograms of differentiation between three isolates of *Leuconostoc mesenteroides* ssp. *mesenteroides* C, D and I, obtained after analysis by Bionumerics

Antifungal activity of bacterial supernatants

The antifungal activity results of the four prepared supernatants are shown in Figure 4. *A. carbonarius*, *Lactococcus lactis* ssp. *lactis* (F) and *Leuconostoc mesenteroides* ssp. *mesenteroides* (I) were found to inhibit fungal radial growth with significant percentages of 53.31% and 50.02%, respectively. The percentage of inhibition by the two strains of *Leuconostoc mesenteroides* ssp. *mesenteroides* (C and D) was moderate, with 33.02% and 28.89% growth inhibition, respectively. Likewise, the growth inhibition of *A. niger* was about 51.39% by *Lactococcus lactis* ssp. *lactis* F, with moderate growth inhibition of 37.45%, 36.61% and 28.69% by the three strains of *Leuconostoc mesenteroides* ssp. *mesenteroides* (I, C, and D) supernatants, respectively.

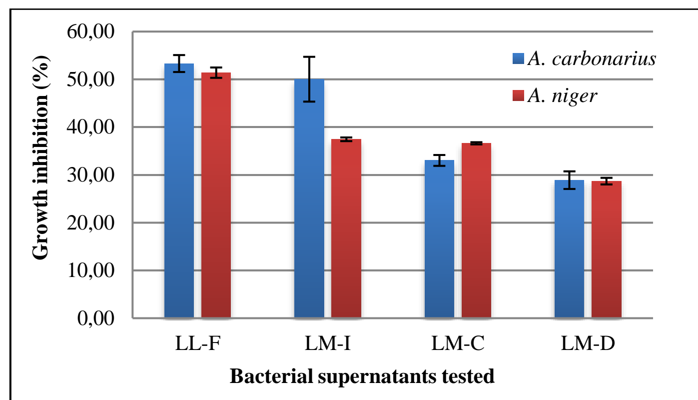


Figure 4 Growth inhibition (%) of *Aspergillus niger* and *A. carbonarius*, in presence of supernatants of *Lactococcus lactis* ssp. *lactis* (LL-F) and three strains of *Lenconostoc mesenteroides* ssp. *mesenteroides* (LM-I, LM-C and LM-D)

The effect of lactic acid bacteria supernatants on OTA production

The concentrations of OTA in the control culture of both *Aspergillus* species are similar, with 96.36 and 97.96 ng/g for *A. niger* and *A. carbonarius*, respectively (Table 3). The effect of the supernatants obtained from *Lactococcus lactis* ssp. *lactis* (F) and *Leuconostoc mesenteroides* ssp. *mesenteroides* (I), which appeared significant percentages of growth inhibition on OTA production by *A. niger* was very important. Indeed, this reduction was around 63.90% and 89.38% for the *Lactococcus lactis* ssp. *lactis* (F) and *Leuconostoc mesenteroides* ssp. *mesenteroides* (I) supernatants, respectively. Concerning *A. carbonarius*, while the reduction percentage of OTA production in the presence of the *Lactococcus lactis* ssp. *lactis* (F) supernatant (65.12%) was similar to that of *A. niger* (63.90 %), it was only 19.35% with the *Leuconostoc mesenteroides* ssp. *mesenteroides* (I) supernatant.

Table 3 Quantification of OTA produced by the *Aspergillus* sp. treated with the supernatants of *Lactococcus lactis* ssp. *lactis* F and *Lenconostoc mesenteroides* ssp. *mesenteroides* I (LOD = 0.2 ppb)

	***Aspergillus niger* (MUCL 49226)**		***Aspergillus carbonarius* (MUCL 49345)**	
	OTA (ng/g agar)	**Reduction of OTA %**	**OTA (ng/g agar)**	**Reduction of OTA %**
Control supernatants	96.36 ± 8.87		97.96 ± 13.47	
Lactococcus lactis ssp. *lactis* (F)	35.08 ± 8.66	63.90 %	34.17 ± 2.84	65.12 %
Leuconostoc mesenteroides ssp. *mesenteroides* (I)	10.23 ± 3.99	89.38 %	79.01 ± 1.85	19.35 %

DISCUSSION

Currently, the control of black mold contamination in vineyards is mainly based on the use of synthetic fungicides; however, the appearance of the secondary effects of these fungicides has called into question their use. Indeed, the appearance of resistant strains, the problems of residues at different stages of product life, and the negative brand image of the products induced by consumer dissatisfaction also justify the search for alternative methods to control, prevent, inactivate, retard, or inhibit growth of these spoilage fungi **(Arfaoui, 2019; Garnier, 2017)**. In this study, the results obtained by qualitative methodology showed that the three *Leuconostoc mesenteroides* ssp. *mesenteroides* strains (I, C and D) and *Lactococcus lactis* ssp. *lactis* (F) inhibited the growth of *A. carbonarius* and *A. niger*. This inhibition can be attributed to the antifungal compounds produced by lactic acid bacteria, such as organic acids, fatty acids, bacteriocins, reuterin, diacetyl, and hydrogen peroxide **(Salas *et al.,* 2017; Crowley *et al.,* 2013; Gerez *et al.,* 2009; Prema *et al.,* 2008; Magnusson *et al.,* 2003; Lavermicocca *et al.,* 2000)**. In addition, the efficiency of the antifungal activity of lactic acid bacteria depends on several factors, including temperature, incubation period, and culture medium used **(Dalié *et al.,* 2010)**. In this work, the antifungal activity observed in four tested strains was detected at a temperature of 30 °C and an incubation period of 48 h. Our results are in agreement with those obtained by different authors who showed that 30 °C was the most adequate temperature for the antifungal activity of different bacterial strains, such as *Lactococcus lactis* ssp. *lactis* **(Roy *et al.,* 1996)**, *Lactobacillus coryniformis* ssp. *coryniformis* **(Magnusson & Schnürer, 2001)**, and *Lactobacillus plantarum* **(Rouse *et al.,* 2008)**. In contrast, **Zhao (2011)** observed that the maximum antifungal activity for *Lactobacillus plantarum* NB and *Lactobacillus plantarum* DC2 was obtained at temperatures of 25 and 37°C. Regarding incubation period, antifungal activity was not detected until after 48 h. This is in accordance with the results of **Dalié (2010)**, which showed that antifungal activity improved with increasing incubation time. This could be related to antifungal compounds, likely secondary metabolites, which are released only during cell lysis when lactic acid bacteria are in a phase of declining growth **(Dalié, 2010)**. We have also shown that antifungal activity of the four strains (F), (I), (C), and (D) was most important in MRS medium compared to M17 medium. According to **Salas *et al.* (2017)**, the composition of MRS medium can have a significant impact on the expression of antifungal activity of lactic acid bacteria. This impact could be related to the presence of sodium acetate in MRS medium, which appears to reinforce antifungal activity. In addition, no effect was observed by using MRS medium as a control to inoculate both *Aspergillus* sp. tested. This shows that sodium acetate alone has no antifungal effect. Our results are in agreement with **Schnürer and Magnusson (2005)**, **Stiles *et al.* (2002)**, and **Delavenne *et al.* (2012)**, confirming that sodium acetate present in MRS medium participates, in synergy with some antifungal compounds produced by lactic acid bacteria, in increasing the inhibitory effect.

Regarding the results of our quantitative methods, the supernatants of four selected isolated strains inhibited growth of both *Aspergillus* sp. studied, with percentages between 28.69% and 51.39%. These results confirm the presence of antifungal compounds in the supernatants. These percentages are similar to those obtained by **Ben Taheur *et al.* (2019)**, which was 37.78% when using the supernatant of *Lactobacillus kefiri* (FR7) against *A. carbonarius*. Conversely, the results obtained by **Le Lay *et al.* (2016)** showed that *Leuconostoc mesenteroides* and *Lactococccus lactis* ssp. *lactis* had no effect on the growth of *A. niger*. This diversity in results agrees with **Russo *et al.* (2017)**, which showed inter-specific variability in the spectrum of antifungal activity, especially with *Lactobacillus plantarum*. In fact, this difference in antifungal activity observed in lactic acid bacteria corresponding to the same species, or even to the same subspecies, suggests genetic diversity in the global genome. The results obtained by PFGE supported this hypothesis. Indeed, the three *Leuconostoc* (I, C and D) studied are different, with high percentages of similarity (Figure 4). It may be suggested that this genetic diversity, while being weak, nevertheless induces strain-dependent inhibitory activity.

In addition to deterioration of the nutritional and organoleptic quality of food, contamination by fungi often induces the formation of mycotoxins. Their presence in food constitutes a danger to human and animal health **(Dalié *et al.,* 2010)**. The most effective way to prevent contamination of food with mycotoxins is to prevent growth of mycotoxigenic fungi **(Varga *et al.,* 2005)**. In this study, we evaluated the ability of lactic acid bacteria to not only inhibit growth of *A. niger* and *A. carbonarius* but also their effects on OTA production. For this, (F) and (I) isolate strains (those with significant percentages of growth inhibition) were selected. To our knowledge, this article is one of the first to study the effect of *Lactococcus lactis* ssp. *lactis* and *Leuconsotoc mesenteroides* ssp. *mesenteroides* on OTA production by *A. niger* and *A. carbonarius*.

In the case of *A. niger*, OTA production was reduced by roughly 89.38% with the use of the *Leuconsotoc mesenteroides* ssp. *mesenteroides* (I) supernatant and 63.90% in the case of *Lactococcus lactis* ssp. *lactis* (F). This reduction may be due to reduced mycelial growth after using bacterial supernatants. Indeed, **Gerbaldo *et al.* (2012)** and **Dallagnol *et al.* (2018)** noted a correlation between fungal growth and production of mycotoxins when using lactic acid bacteria. Thus, in our case, the growth inhibition observed may have contributed to this weak synthesis of mycotoxins; however, this correlation is not always effective **(Dallagnol *et al.,* 2018)**. Furthermore, this reduction of OTA production may also be linked to antifungal compounds produced by the lactic acid bacteria studied. Indeed, it is known that some antifungal compounds, especially organic acids, can diffuse through the membrane of target organisms in their undissociated hydrophobic form. In the cytoplasm, due to its neutrality, acid will dissociate and generate an accumulation of protons, thereby reducing intracellular pH. This generally causes inhibition of key enzymes of metabolism, inducing a loss of viability and cell destruction **(Dalié *et al.,* 2010; Le Lay, 2015; Lappa *et al.,* 2018)**. By using the supernatant of *Leuconsotoc mesenteroides* ssp. *mesenteroides* (I), we found the percentage reduction of OTA production by *A. carbonarius* is only 19%. It is possible that *A. carbonarius* has developed resistant bacterial antagonism; however, this resistance is only observed in the presence of *Leuconsotoc mesenteroides* ssp. *mesenteroides* (I) and not with *Lactococcus lactis* ssp. *lactis* (F). This suggests the existence of other antifungal compounds involved in the reduction of OTA that are produced only by the *Lactococcus lactis* ssp. *lactis* (F) strain. This agrees with the work of **Sadeghi *et al.* (2019)**, which showed that the antifungal activity of lactic acid bacteria is linked to the nature and quantity of the inhibitory compounds they produce.

Our study indicates that the selected strains of lactic acid bacteria can be applied as a biocontrol of fungi growth and mycotoxins production in food product. In fact, several studies showed that strains with *in vitro* antifungal activity were found to be either less or more active in food products. In general, this can be achieved either by adding the bacterial supernatant, purified antifungal compounds, or whole bacteria as active ingredients, or by spraying them into the surface of an intact fruit or wound, prior to inoculation of the fungal target **(Le Lay *et al.,* 2016; Ben Taheur *et al*., 2019)**.

CONCLUSION

This study demonstrated that four selected strains of lactic acid bacteria isolated from different fermented Moroccan products, are able to inhibit the growth of *A. niger* and *A. carbonarius*, with *Lactococcus lactis* ssp. *lactis* (F) and *Leuconostoc mesenteroides* ssp. *mesenteroides* (I) being the most active. Indeed, these two strains of lactic acid bacteria also reduced the production of OTA as indicated by high percentages. Nevertheless, *Lactococcus lactis* ssp. *lactis* (F) was able to reduce OTA production by *A. carbonarius* strain (65.12%). In contrast, *Leuconostoc mesenteroides* ssp. *mesenteroides* (I) was proved less efficient to inhibit OTA production from the same fungal strain (19.35%). In this regard, we suggest a resistance mechanism of fungal isolate upon bacterial antagonism. As such, the use of lactic acid bacteria as an alternative against fungal alterations would be more effective in the presence of a mixture of strains in order to avoid resistance mechanisms.

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