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ANTIFUNGAL POTENTIAL OF LACTIC BACTERIA FROM TRADITIONAL SOURDOUGH STARTERS FOR FOOD PRESERVATION

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Abstract. The objectives of this study are to investigate the antifungal activity of lactic acid bacteria isolated from four samples of bread sourdough prepared using traditional methods, made from flour and sterile water, fermented goat's milk, lemon juice, with three chopped garlic cloves added to one sample. 78 lactic isolates are isolated, 63% rod-shaped and 37 % cocci, with positive Gram and negative catalase. The antifungal activity of these lactic isolates was assessed *in vitro* by two methods: a direct confrontation test with *Fusarium globosum* and a double-layer diffusion test against four antagonistic fungi: *Aspergillus niger*, *Alternaria angustiovoidea*, *Aspergillus flavus* and *Fusarium globosum*, previously isolated from contaminated vegetables. The first screening results showed that 24.35 % of the lactic isolates had antifungal activity. While with the second test, we have identified six strains that were more active against molds, strains BL03 and BL49 are the most active, strains BL11 and BL43 showed moderate activity and strain BL56 is the least active. The phenotypic characterization of the six most active lactic bacteria was confirmed by molecular identification, particularly 16S rDNA sequencing and MALDI-TOF mass spectrometry. The bacterial isolates BL03 and BL49 are of particular interest for their antifungal potential, and this study aims to characterize their mode of action and assess their efficacy in a biopreservation context.

Key words: Lactic acid bacteria; antifungal activity; direct confrontation test; diffusion test; 16S rDNA sequencing, MALDI-TOF mass spectrometry.

INTRODUCTION

Food spoilage is a major problem for the food industry, resulting in food waste, substantial economic losses for manufacturers and consumers, and a negative brand impact. Among the causes, fungal contamination can be encountered at different stages of the food chain (e.g., post-harvest, during processing, or storage) [1]. Fungi can synthesize mycotoxins, toxic fungal secondary metabolites such as aflatoxins, ochratoxins, and fumonisins, which pose a significant risk to food safety [2]. Mycotoxins contamination of agricultural crops represents a major risk for international food protection. More than 25% of cereals and almost 20% of plant production are expected to be affected, which could result in economic losses of up to 60 billion dollars [3]. In order to extend the shelf life of products and prevent microbiological spoilage, the food industry uses a variety of treatments, including fungicides and chemical preservatives. However, current regulations are increasingly encouraging a reduction in the use of these compounds in favor of natural preservation methods [4]. Promising studies are exploring the potential of certain indigenous microorganisms, traditionally used in fermentation, to inhibit the production of mycotoxins or neutralize their harmful effects [5].Lactic acid bacteria have traditionally been used in food fermentation processes and are recognized for their various antimicrobial properties while preserving the sensory qualities of food [6].

Lactic acid bacteria are Gram-positive immobile microorganisms, lack catalase and endospores. They are characterized by their fermentative metabolism, which focuses on producing lactic acid. This microbial family is subdivided into two physiological groups: homofermentative and heterofermentative lactic acid bacteria, giving rise to various metabolites. In addition, their ability to synthesize antimicrobial substances makes them key players in food preservation [7]. Their scientific interest has grown in recent years, not least because of their antifungal properties [8]. They are considered the best candidates for protecting a wide range of food products against spoilage fungi [6].

MATERIALS AND METHODS

The practical part of our work was carried out in the Natural Saharan Resources (RNS) laboratory at the Ahmed Draia University in Adrar and Molecular Microbiology Proteomics and Health (MMPS) laboratory at the Djillali Liabès University in Sidi Bel Abbés.

Reconstitution of bread ferments

The various traditional sourdoughs are prepared from basic ingredients such as wholemeal flour, sterile water and other starting agents such as lemon juice, fermented goat's milk or garlic, then mixed with a glass rod in a sterile bottle. The leavens prepared according to the four procedures described below were incubated at 28°C for 24 hours according to the traditional methods established by Faid et *al*., 1994 [9] . Successive additions of wholemeal flour and sterile water fermented them. We then incubated them at 28°C until the pasta had risen. The preparation conditions explained in the table below:

Table 1. The various traditional preparations of bread sourdoughs

Samples	**Method of preparation**
Sourdough I	50 ml warm water + 100 g wholemeal flour.
Sourdough II	50 ml of goat's milk fermented to pH =3.82 was mixed with 100 g of wholemeal flour.
Sourdough III	10 ml lemon juice and 40 ml water + 100 g wholemeal flour.
Sourdough IV	100 g of flour + 50 ml of water, and three cloves of garlic were chopped and added to the mixture.

Isolation and purification of lactic bacteria isolates

Isolation of lactic acid bacteria was carried out using the different sourdoughs. 1 ml of the different dilutions (10^{-1}, 10^{-2} and 10^{-3}) were inoculated into petri dishes containing MRS agar medium. Incubation occurred anaerobically in a jar under a CO_2+N_2 atmosphere (GasPak system, BBL) at 30°C for 48 to 72 hours. After purification by successive subculturing, the isolates were phenotypically characterized by Gram staining, catalase test and fermentation test (homolactic/heterolactic). Pure strains were stored at 4°C.

Isolation and purification of the fungal species

Fungal species associated with fruit and vegetable spoilage, samples of tomatoes, cucumbers, and zucchini from the Talmine region (Adrar) were subjected to microbiological determination. Fragments weighing 1g were macerated in sterile water, inoculated onto PDA agar, and incubated at 25°C for 7 days. Colonies developed around plant tissues were purified by successive subcultures [10].

Morphological identification of fungal isolates

The fungal isolates were identified based on macroscopic examination of the colonies (cultural characters) and microscopic examination of the thallus and reproductive structures (sexual and asexual) on the PDA medium. The identification keys of Barnett and Hunter (1998) [11] were used as a reference to classify isolates into different genera.

Molecular identification of fungal isolates

Genomic DNA was extracted from 7-day-old fungal cultures following the protocol described by Chamekh et *al*. (2019) [12]. Two regions were amplified according to the genera identified. The ITS1-5.8S-ITS2 (Internal Transcribed Spacer) region of the DNAr was amplified for strains belonging to the genera Aspergillus and Alternaria using primers ITS4 and ITS5 [13] and the translation elongation factor 1α (TEF-1α) gene for strains belonging to the genus Fusarium using primers EF1F / EF1R [14]. Molecular determination (extraction, amplification, and sequencing) was carried out by the Gene Life Science laboratory (Sidi Bel Abbés).

Antifungal activity of lactic bacteria isolates

Antifungal lactic acid bacteria were selected using two methods: the confrontation method, which allows the zones of inhibition to be observed visually, and the double layer method, which offers a more precise quantification of antifungal activity. The confrontation method described by Gerbaldo et *al*. 2012 [15] is adopted with some modifications. Lactic bacteria strains were inoculated in 2 cm lines in Petri dishes containing MRS medium and incubated anaerobically in a CO_2+N_2 atmosphere (GasPak system, BBL). After a pre-incubation period of 48 hours at 30°C to allow lactic acid bacteria to develop, a fragment of *Fusarium globosum* was placed in the centre of the agar. Incubation was continued for 3 to 5 days under the same conditions. A negative control containing only *Fusaruim globosum* was also prepared for comparison. After incubation, the fungi development remained limited between the two lines of an active lactic acid bacteria (antifungal activity), otherwise for the inactive lactic acid bacteria or in the control dish, the fungi species developed over most or all of the petri dish. For the double-layer method, the antifungal activity of the lactic strains was determined using the recovery method described by Magnusson et *al*. 2003[16], with a few modifications. Bacteria were inoculated two lines wide onto MRS agar plates and incubated anaerobically. After pre-incubation for 48 hours at 30°C, the plates were coated with 10 ml of 0.8 % PDA containing 10^6 spores of the antagonistic strains *Aspergillus niger*, *Alternaria angustiovoidea*, *Aspergillus flavus* and

Fusarium globosum incubated for 3 to 5 days at 30°C. A negative control was also prepared without lactic acid bacteria and with molds only.

The results are interpreted as follows:

- Bacteria with no zone of inhibition are considered inactive bacteria (-);
- Less active bacteria (+) : zone of inhibition between 0.1 and 3 % of the surface of the plates;
- Active bacteria (++): zone of inhibition between 3 and 8 % of the surface of the plates;
- Very active bacteria (+++): zone of inhibition greater than 8 % of the surface of the plates.

Phenotypic characterization of antifungal lactic isolates

Phenotypic characterization of antifungal lactic isolates is based on morphological (macroscopic and microscopic), biochemical (type of fermentation, catalase, arginine dihydrolase and citrate) and physiological (growth at different temperatures (15°C and 45°C) and NaCl concentration (4% and 6.5%)) studies [17].

Identification of antifungal strains by 16S rDNA gene sequencing and MALDI BRUKER

The antifungal lactic isolates were identified by 16S DNAr gene sequencing and MALDI Bruker at the International Microbial Resource Center for Bacteria of Food Interest (CIRM) in France. The DNA of our selected antifungal isolates was extracted using the Macherey Nagel 'Nucleospin Tissue' Kit according to Gordon et *al*.1997 [18] extraction method. The 16S DNAr fragments were amplified by PCR. The amplicons obtained were purified and sequenced using Sanger et *al*.1997 [19] technique. The obtained sequences were compared with those referenced in the NCBI database using Geneious software (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Young colonies less than 48 h old from each isolate were deposited on a spot and coated with HCCA matrix (Bruker reference) for the BRUKER MALDI technique. After drying, a BTS control was added (Bacteria Test Standart, Bruker Reference) to validate the run. The plate was inserted into the instrument for identification by the MALDI Biotyper® Sirius One (Bruker). Analysis was performed using MBT Compass HT software.

Production of antifungal substances

Two of the most potent lactic strains, BL03 and BL49, were evaluated for their ability to synthesize active antifungal substances in MRS broth. The 18 h young lactic culture was inoculated into 600 ml MRS broth (1%, v/v) and incubated at 30°C for 72 h under anaerobic conditions. Aliquots were taken initially at 6 h intervals, then at 24 h intervals at the end of the

exponential growth phase. They were analyzed for pH, cell growth (optical density at 600 nm) and antifungal activity of the cell-free supernatant. Sathe et *al*. 2007[1] described the method to obtain the cell-free supernatant. The bacterial culture was centrifuged at 10 000 g for 10 minutes, then the supernatant was filtered through 0.22 mm filters (Millipore SA), the extract obtained was used to determine antifungal activity.

A 2 ml aliquot of the extract was incorporated into 15 ml of PDA and then aseptically poured into a Petri dish, after drying, 3 ul of mold spore suspension (10^6 spores/ml) was inoculated on the agar, which were incubated at 25°C for 5 days. The diameter of the fungal colony was measured and compared with a negative control [20].

Antifungal activity was assessed by measuring the zone of inhibition of fungal colony growth, calculated using the formula I = 1- (D S/D C). DS represents the colony diameter for the sample, while DC represents the diameter of the control colony.

In vivo testing of fungal deterioration in vegetables

This test was carried out on surface-sterilized plant material that had undergone a standardized wound using a cork borer ($2\times2\times2mm^3$). The wounds were inoculated with 20 µl (10^4 cells ml-1) of lactic strains followed by an inoculation of 20 µl of a spore suspension containing 10^4 spores ml^{-1} of the antagonistic strains Aspergillus Niger, Alternaria angustiovoidea, Aspergillus flavus and Fusarium globosum. The controls were inoculated with fungal agents or with lactic strains. Lesion diameters were measured 9 days after inoculation in a controlled atmosphere at 20°C and 85 % relative humidity [1].

Statistical analysis

The data represent the mean of three replicates ± standard deviation (SD). Results were subjected to multiway analysis of variance, and the mean comparisons were performed by RProject version 4.4.2 (Statistical Package for R-Foundation). Differences between means were considered significant at p-value < 0.05.

RESULTS Identification of fungal isolates

The mycological determination of five vegetables contaminated by fungi revealed the presence of 31 distinct fungal isolates, isolated and purified on PDA medium. The morphological study identified the genera *Aspergillus*, *Fusarium* and *Alternaria*, accounting for 46.88 %, 28.12 % and 21.88 % of the isolates, respectively. These results suggest that these three genera play a major role in the fungal contamination of the vegetables studied (see Table 2).

Table 2. Number and frequency of fungal strains isolated.

Genre	Number	Frequency
Aspergillus	15	46.88%
Fusarium	09	28.12%
Alternaria	07	21.88%

The molecular study identified the species of the four fungal strains used in this study as antagonistic agents: *Fusarium globosum*, *Aspergillus niger*, *Alternaria angustiovoidea* and *Aspergillus flavus* (Table 3).

Table 3. List of fungal antagonist isolates and their closest match with the NCBI GenBank database.

Code of strains	Locus	Closest match in GenBank	Maximum coverage of the identifier/query
SF06	ITS	*Aspergillus niger*	100 /100
SF14	ITS	*Alternaria angustiovoidea*	98/100
SF22	ITS	*Aspergillus flavus*	99/100
SF27	TEF	*Fusarium globosum*	100/100

Isolation and screening of lactic acid bacteria for antifungal activity

A total of 78 strains were isolated and purified on MRS agar from the four leavens prepared. All isolates showed a morphology typical of lactic acid bacteria: catalase-negative and Gram-positive. Microscopic observation showed a predominance of rod forms (63%) compared with coccus forms (37%).

The confrontation test was used to select 19 lactic isolates with antifungal potential against *Fusarium globosum*. The results of the double-layer screening showed that 19 antagonistic strains stood out for their strong, moderate and weak antifungal capacity against the fungi *Aspergillus niger*, *Alternaria angustiovoidea*, *Aspergillus flavus* and *Fusarium globosum*. The two isolates, BL03 and BL49, have a broad spectrum of action against all antagonistic agents, unlike other lactic strains, such as BL33 and BL56, which showed low activity (see Figure 1). The double-layer results (qualitative test) are presented in Table 4.

Figure 1. *Isolates BL03 and BL05 demonstrated strong activity (+++) against Aspergillus niger.*

Table 4. Results of double-layer screening of the antifungal activity of lactic isolates.

Origin	Isolates lactic	Fungal targets			
		Fusarium globosum	***Aspergillus flavus***	***Aspergillus niger***	***Alternaria angustiovoidea***
Leaven I	**BL01**	++	++	-	++
	BL03	+++	+++	+++	++
	BL05	++	+++	+++	++
	BL06	+	-	-	++
	BL07	+++	++	++	+++
	BL09	++	+++	+++	++
	BL11	++	++	++	+
Leaven II	**BL26**	+	+	-	+
	BL29	+	+	+	-
	BL30	+	+	+	-
	BL33	+	-	+	+
	BL35	+++	+++	++	++
	BL41	+	+	-	++
	BL43	++	++	++	+
Leaven	**BL47**	+	++	+	-

III	**BL49**	++	+++	+++	+++
Leaven IV	**BL50**	++	+	+	-
	BL56	+	-	-	+
	BL72	++	-	-	+

The inhibition zones are classified as follows: (-) no inhibition zone; (+) inhibition zone between 0.1 and 3 % of the surface area of the dish; (++) inhibition zone between 3 and 8 % of the surface of the dish and (+++) inhibition zone greater than 8 %.

Phenotypic characterisation of antifungal lactic isolates

The six lactic bacteria isolates with the best antifungal activity were subjected to phenotypic identification (or characterisation) (Table 5), all isolates were catalase-negative, gram-positive. Five of the isolates were short chain bacilli in pairs. Only one isolate was coccus (BL07).

Table 5. Physiological and biochemical characteristics of antifungal lactic isolates.

N°Strain	**Catalase**	**Gram reaction**	**Observations ×100**	**Type fermentative**	**ADH**	**Citrate**	**T°C**		**NaCl%**	
							15	**45**	**4**	**6.5**
BL03	_	+	Short bacilli in pairs or chain	Facultatively heterofermentative	-	+	+	-	+	+
BL05	_	+	Short bacilli in pairs or chain	Facultatively heterofermentative	-	+	+	-	+	+
BL07	_	+	Hulls in pairs or tetrads	Homofermentative	+	-	-	+	+	+
BL09	_	+	Short bacilli in pairs or chain	Facultatively heterofermentative	-	+	+	-	+	+
BL35	_	+	Short bacilli in pairs or chain	Facultatively heterofermentative	-	+	+	-	+	+

BL49	_	+	Short bacilli in pairs or chain	Facultatively heterofermentative	-	+	+	-	+	+

Identification of antifungal strains by 16S rDNA gene sequencing and Bruker MALDI

The strains BL03, BL05, BL09, and BL49 belong to the Lactiplantibacillus genus. With a 99.9 % identification rate, two species were suggested using the 16S rDNA sequencing method, namely *L. argentoratensis* and *L. plantarum.* The three strains BL03, BL05, and BL49 were identified as *Lactiplantibacillus plantarum* using Bruker MALDI mass spectrophotometry. Strain BL09, however, was not identified. With a 99.8 % identification rate, strain BL35 revealed two species: *L. plantarum* and *L. pentosus*, but with Bruker MALDI technique, this strain is a *Lactiplantibacillus plantarum*. Both techniques were used to identify strain BL07 as *Pediococcus pentosaceus* (Table 6).

Table 6. Identification by 16S rDNA sequencing and Bruker MALDI mass spectrophotometry.

N° Strain	**Identification by 16S rDNA sequencing**	**% of identification**	**Identification by MALDI Bruker**	**Score of identification**
BL03	*Lactiplantibacillus plantarum/ argentoratensis*	**99.9%**	*Lactiplantibacillus plantarum*	**2.36**
BL05	*Lactiplantibacillus plantarum/ argentoratensis*	**99.9%**	*Lactiplantibacillus plantarum*	**2.34**
BL07	*Pediococcus pentosaceus*	**99.9%**	*Pediococcus pentosaceus*	**2.24**
BL09	*Lactiplantibacillus plantarum/ argentoratensis*	**99.9%**	No spectrum obtained	**-**

BL35	*Lactiplantibacillus plantarum/ pentosus*	**99.8%**	*Lactiplantibacillus plantarum*	**2.13**
BL49	*Lactiplantibacillus plantarum/ argentoratensis*	**99.8%**	*Lactiplantibacillus plantarum*	**2.38**

Production of antifungal substances

The pH and cell density (OD) of each aliquot taken at a specific time during growth (72 hours) of the two strains BL03 (Lactiplantibacillus plantarum) and BL49 (Lactiplantibacillus plantarum) in MRS broth at 30°C were measured, as shown in Figures 2 and 3. In parallel, the antifungal activity of the metabolites produced was assessed, and the results are presented in Figure 4.

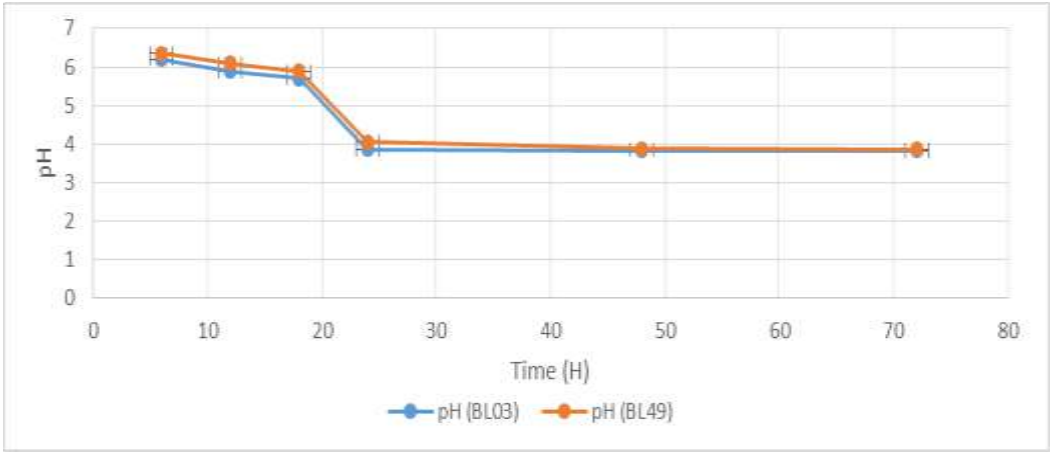

Figure 2. *Changes in pH of L. Plantarum (BL03) and L. Plantarum (BL49) strains in MRS broth at 30°C over 72 h.*

Measurement of the pH of aliquots taken from the MRS medium inoculated with two lactic acid bacteria, BL03 and BL49, showed strong acidification, with the pH dropping from 6.62 to 3.38 for BL03 and from 6.38 to 3.88 for BL49. However, an increase in cell density was observed for both bacteria.

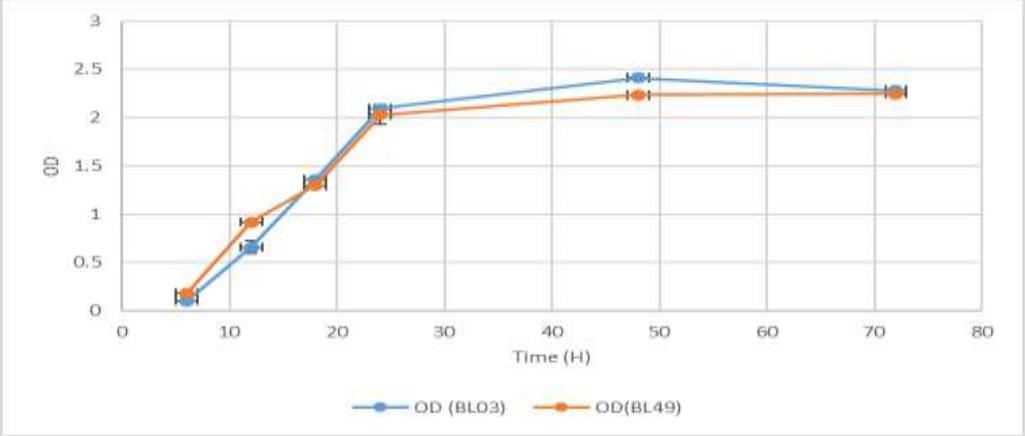

Figure 3. *Cell density of L. Plantarum (BL03) and L. Plantarum (BL49) strains in MRS broth at 30°C for 72 hours.*

Biostatistical tests between pH and OD were carried out with a significance level of 5% (0.05). A correlation between pH and OD was observed for the BL03 and BL49 lactic strains. The correlation coefficient for the BL03 lactic acid bacterium was -0.9495899 with a p-value equal to 1.789e-09. The correlation coefficient for the BL49 lactic acid bacterium was -0.94969179 with a p-value equal to 2.682e-09.

Analysis of the correlation coefficient for the two lactic acid bacteria reveals a p-value << 0.05, indicating that this coefficient is significantly different from zero. So, at a 95% confidence interval for the correlation coefficient, we conclude a strong negative correlation between pH and OD.

The antifungal activity increased progressively with the incubation period up to 48 hours. It reached a peak of optimal activity in the late exponential and late stationary phases. However, a significant decrease in activity was observed after 48 hours.

Figure 4. *Evaluation of the antifungal activity of secondary metabolites of L. Plantarum (BL03) Vs Aspergillus niger strains.*

These results suggest the presence of secondary metabolites, particularly organic acids, which could be responsible for this activity. Statistical tests between pH and inhibition (antagonistic activity) were carried out with a significance level of 5% (0.05). Table 7 shows the correlation between pH and inhibition.

Table 7. Correlation coefficients between pH and inhibition.

	ISF27	**ISF22**	**ISF06**	**ISF14**
pH (BL03)	-0.9082520	-0.8761313	-0.8625555	-0.8689122
pH (BL49)	-0.9068652	-0.8677228	-0.8407856	-0.9184562

Comparison of the inhibition of the two lactic acid bacteria BL03 and BL49

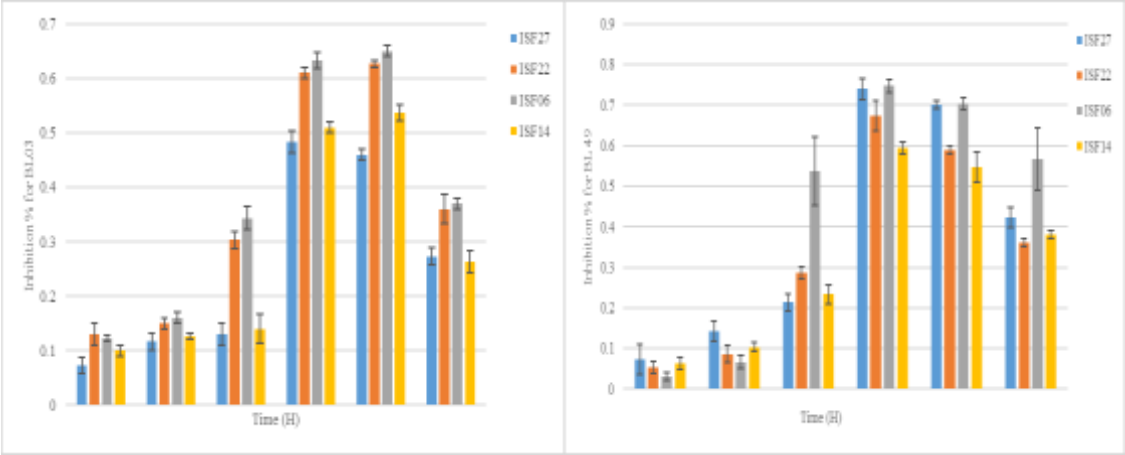

Figure 5. *Production of antifungal substances by L. Plantarum (BL03) and L. Plantarum (BL49) strains.*

The size of our sample is less than 30 (n = 18), so after testing the normality of the distribution (Shapiro-Wilk test), it is sufficient to carry out the test on the BL03 bacteria: the p-values are between 0.0015 and 0.0085, p-values < 0.05, so the tests are, therefore, significant

(absence of normality). The alternative non-parametric test (Mann-Whitney test) was then performed to compare the inhibitions of the two bacteria.

The p-values of the comparison between the inhibitions of the two lactic bacteria on the four fungi species are comprise between 0.2311 and 0.6718, so all p-values > 0.05, the test is not significant, there is no tested significant difference between the two averages, and two bacteria exert similar inhibitions on the fungal strains. **In vivo testing of fungal deterioration in vegetables**

The plant material used for the in vivo test was in perfect condition when exposed separately to these four fungi with the presence of the two strains of lactic bacteria BL03 and BL49, or without lactic bacteria as control. The results presented in Table 7 indicate that the use of the L. plantarum (BL03) and *L. plantarum* (BL49) strains slowdown significatively the decomposition of vegetables caused by the fungi *Fusarium globosum*, *Aspergillus niger*, *Alternaria angustiovoidea* and *Aspergillus flavus*.

Table 10. Diameter of lesions (mm) on vegetables subjected to different spoilage fungi and on wounds inoculated with *L. Plantarum (BL03)* or *L. Plantarum (BL49)*.

Fungal Strains	**Cucumbers**			**Courgettes**			**Tomatoes**		
	BL 03	**BL 49**	**Control Pathogen**	**BL 03**	**BL 49**	**Control Pathogen**	**BL 03**	**BL 49**	**Control Pathogen**
Aspergillus niger	5	25	55	2	35	50	10	15	30
Aspergillus flavus	8	15	18	1	25	40	8	11	35
Fusarium globosum	2	2	10	12	25	30	13	2	40
Alternaria angustiovoid	12	18	35	15	15	29	5	13	25

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DISCUSSION

Sourdough fermentation is a traditional food processing method based on cereal flour fermentation [21]. Each sourdough is determined by a microbial ecosystem composed of yeasts and lactic bacteria whose metabolic activities are influenced by various physicalchemical and environmental parameters [22]. The study carried out by Faid et *al*. in 1994 highlighted the biodiversity of lactic acid bacteria found in homemade sourdoughs. Researchers have recently used various cereal sourdoughs as a source of antifungal lactic acid bacteria [23, 24]. As part of this research, 78 lactic acid bacteria were isolated from traditional bread sourdoughs to identify those with antifungal properties against fungal spoilage strains isolated from contaminated vegetables and examine their potential ability to ensure the biological preservation of vegetables and fruit. Six lactic strains that had demonstrated efficacy against the four fungi were selected for phenotypic and molecular characterisation, revealing that most strains belonged to *Lactiplantibacillus plantarum*. Our results agree with the results reported by De Simone et *al*. 2021[25], who isolated powerful antifungal lactic acid bacteria from bread leavens. They were all identified as *Lactiplantibacillus plantarum*. The acidification of the cell-free supernatant resulting from the metabolism of lactic bacteria is responsible for their antifungal activity. Researchers explain that the organic acids (secondary metabolites) synthesized by lactic bacteria contribute to antifungal activity [26, 27]. The organic acids produced by the *L.plantarum* strain come from the facultative heterofermentative metabolism of carbohydrates, which synthesizes lactic acid with other products, and from the metabolism of amino acids; for example, leucine is metabolized into 2hydroxyisocaproic acid (HICA) [28,8]. Antifungal activity values correlated with cell density, peaking during the exceptional growth phase and decreasing during the stationary phase. These results suggest that the antifungal compound may undergo transformation into other metabolites or be degraded by autolytic enzymes [1]. The two *L. plantarum* strains, BL03 and BL49, although of different origins, showed similar inhibition capacities on the four fungal strains tested. The study by de Crowley et *al*.2013 [29] highlights the efficacy of *L.plantarum* species, isolated from various animal, plant and human samples, as broad-spectrum antifungal agents. According to the results obtained, in vivo tests of the BL03 and BL49 bacterial strains demonstrated their ability to extend the shelf life of vegetables by significantly reducing contamination levels. Other research has shown that lactic

acid bacteria have the ability to delay fungal spoilage in cucumbers [1], apples [30], grapes and plums [29].

CONCLUSION

Lactic acid bacteria have demonstrated antifungal characteristics, making them potential agents for controlling fungal growth in the food industry, including the long-term preservation of vegetables and fruit. The potential advantages of using lactic strains as antifungal agents are manifold. Firstly, they are generally considered beneficial for human consumption, making them a natural alternative to chemical preservatives. They are also relatively simple and inexpensive to produce on a large scale, making them easy to use in the food industry. Finally, they have a broad spectrum of antifungal activity, enabling them to combat different types of pathogenic fungi.

Conflict of Interest There are no real or potential conflicts of interest in relation to this article.

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