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Isolation, characterization and identification of lactic acid bacteria with antimicrobial activities found in fresh pulp of tomatoes from Ouagadougou, Burkina Faso

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Abstract

The objective of this investigation was to isolate, characterize and identify lactic acid bacteria (LAB) from local tomato fresh pulps. A batch of 30 samples of Mongal cultivar tomato purchased at two markets in Ouagadougou, Burkina Faso was used as a matrix for the collection of LAB isolates. From a preliminary biochemical characterization, 97 isolates were collected based on their antimicrobial activity. Seventeen isolates (17.52 % of the isolates collected) displayed antimicrobial activities with an inhibitory zone higher than or equal to 12 mm of diameter, against one to three out to 16 pathogenic indicator strains used including bacteria, moulds and yeasts. Based on their phenotypic characteristics, the 17 isolates were regrouped and 12 representative isolates were used for the identifiation by 16S rDNA sequencing. The results showed that the 17 LAB isolates displaying antimicrobial activity, were capable of fermenting various carbohydrates, and three of them were capable of producing bacteriocin-like substances (BLS) against *Salmonella infantis* SKN 557 (2 isolates) and *Pseudomonas aeruginosa* ATCC (1 isolate). Fourtheen isolates showed inhibitory activity against both *A. flavus* and *A. fumugatus*.The isolates identified belong to the species *Lactobacillus plantarum* (50% of the isolates), *Lactobacillus fermentum* (25%), *Lactobacillus pentosus* (8.33%), *Lactobacillus plantarum/pentosus* (8.33%) and *Pediococcus acidilactici* (8.33%) with a dominance of *Lactobacillus* genus which represents 91% of the identified isolates against 9% of *Pediococus* genus. The isolates revealed interesting properties and shoud be potential candidates to be used as starter cultures for food fermentation and preservation including fruits and vegetables products.

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Introduction

Fruits and vegetables are hardly conservable, causing important post-harvest losses according to the crop and to environmental conditions. According to the ECOWAS report (2011), the lack of adequate post-harvest techniques and the low level of processing cause losses that can often reach 50% of the production. Some fruits and vegetables are consumed as they are or processed into various food products. Mainly fruits and vegetables processes use food additives as preservatives and acidifiers in order to lower the pH, to enhance the taste and to increase the self-life of the final products (Samadoulougou-Kafando *et al.*, 2018). Nowadays, consumers are looking for natural products without chemical additives in order to preserve their health. From this perspective, the use of these chemical acidifiers and preservatives can be substituted by lactic fermentation step with strains of LAB suitably selected and adapted to the raw material. LAB are a group of heterogeneous microorganisms whose main characteristic is the production of lactic acid from fermentation of carbohydrates. They are sought in various substrates and environments for their food applications (Khemariya *et al.*, 2017; Tapsoba *et al.*, 2017) nutritional properties (Fessard, 2017), probiotiques properties (Somashekaraiah *et al.*, 2019) and for bacteriocins production (Ren *et al.,* 2018). In the food industry, LAB are widely used as starter cultures in fermentation processes and in food preservation (Demir *et al.*, 2006). Studies have shown that autochthonous starter cultures ensure better nutritional, organoleptic and sanitary quality of the fermented products compared to allochthonous starter cultures (Di Cagno *et al.*, 2013). Thus, for a fermentation of fruit and vegetable products, LAB from the same ecological niche would be appropriate.

Solanum lycopersicum L. (tomato) is widely consumed crops in Burkina Faso. Indeed, it is the second most important vegetable crops after onion (MASA, 2013). Tomato contains important level of water, minerals (Ca, Fe, K, Mg, Na) and vitamins including pro-vitamin A, vitamins C and B (Hernandez *et al.,* 2008; FAO, 2012) and other bioactive compounds like as antioxydants mainly the lycopene. In 2007, the World Cancer Research Fund (WCRF) Global Network concluded that there is sufficient evidence for the protective effects of foods containing lycopene, in particular tomatoes and their derivatives in prostate cancer through cohort studies and control cases. Tomato products are generally tomato concentrates, tomato paste, tomato gravy, jam, juice and dried tomatoes. It is also contains carbohydrates (amidon, pectine, fructose, glucose) and proteins. Tomato as well as most of fruits and vegetables, provides favourable conditions for the development of LAB. Indeed, LAB have been isolated from fruits and vegetables such as cucumbers, kiwi, olives, papaya, cabbages, carrots, courgettes, eggplants, green beans, red beets, pineapples, plums, fennels, cherries (Plenghvidhya *et al.*, 2007; Di Cagno *et al.*, 2009 Lee *et al.*, 2011). Internationally, LAB of the genera *Lactobacillus, Wessela, Enterococcus, Pediococcus and Leuconostoc* have already been isolated from tomatoes (Di Cagno *et al.*, 2009; Fessard *et al.*, 2017).To our knowledge, no study has been carried out or published on LAB isolated from tomato in Burkina Faso. Characterization of LAB found in tomato fresh pulp from Burkina Faso could harbour isolates with not only particular phenotypes, genotypes but also ecotypes with technological potentials. The present study aims to investigate and identify LAB with potentials functional properties to be used as starter cultures for food fermentation.

Materials and methods

Sampling and preparation of tomatoes samples

A total of 30 samples of ripe and healthy tomatoes were used for the investigation. Mongal cultivar tomato was purchased at two markets (ZOGONA and NABIYAAR) in the city of Ouagadougou, Burkina Faso. In each market, tomatoes were purchased from three vendors based on 5 samples per vendor. Each sample was made up with two tomatoes. In each market, the samples were packaged in plastic freezer bags, placed in a cooler containing carboglaces and transported to the microbiological laboratory of the Institute of Research in Applied Sciences and Technologies (IRSAT) where they were prepared and analyzed within 24 h. The samples of tomato were

washed and well rinsed with potable water to remove external impurities. They were then peeled and cut into small pieces by using sterile gloves, sterilized peeler and stainless steel knives. The tomato pulp of each sample was packaged in a sterile stomacher bag. The peeling and cutting were carried out under a laminar flow hood (Biobase).

Isolation and purification of lactic acid bacteria (LAB)

LAB were isolated on Man Rogosa Sharpe (MRS) medium using the method described by Di Cagno *et al.*, (2011). For each sample, 50 g of mango pulp were suspended in 50 mL of sterile MRS broth (Liofilchem, Italy) used as an enrichment medium and incubated at 30°C for 72 h. After enrichment, dilutions were carried out according to ISO 6887-1 (2017). Inoculation was done in the mass with MRS agar (Liofilchem, Italy). The inoculated petri dishes were placed in anaerobic jars (Biolab) with anaerobic CO_2 generators (Merck KGaA) and were hermetically sealed and incubated in an oven (Binder) at 37°C for 48 h. Plates containing 15 to 150 isolated colonies were selected for isolation. On each of the selected plates, 10 characteristic and distinct colonies were isolated and purified by sequential inoculation of a single colony into MRS broth followed by streaking on MRS agar until pure colonies were obtained. Colony purity was verified by visual observation of colony morphology, size and appearance and by microscopic observation. The purified isolates were introduced into cryotubes containing MRS broth (Liofilchem, Italy) with 30% glycerol (87%) and stored in a freezer (Fiocchetti) at -20°C for further analyses.

Phenotypic characterization of the LAB isolates

The isolates stored in the freezer (-20°C) were revivified on MRS agar (Liofilchem, Italy) for the various tests. Presumptive identification of the isolates was performed using morphological and physiological tests. To select the characteristic isolates for further characterization, the Gram test was performed by the 3% (w/v) KOH method (Gregersen, 1978); the catalase test was performed with hydrogen peroxide (H_2O_2); the thermal shock sporulation test of each isolate was performed in a test tube containing 10 mL of MRS broth (Liofilchem, Italy), and then after incubation at 37°C for 24 h, the tube containing the young cultures was placed in boiled water for 10 min. An aliquot (1 mL) of the treated culture was transferred to a tube containing a sterile MRS broth (Liofilchem, Italy). This tube was then incubated at 37°C and its growth was monitored for 72 h. Growth of the isolate indicates sporulation and absence of growth indicates absence of sporulation. The test for organic acid production consisted of inoculating the isolates in MRS agar (Liofilchem, Italy) containing bromocresol purple BCP (Sigma-Aldrich, Germany) at 0.004% as colored indicator and carbonate calcium (Sigma-Aldrich, Germany) at 1%. After incubation at 37°C for 48 h, acid-producing bacteria appeared yellow on the culture medium which was initially purple (Khemariya *et al.*, 2017). The selected isolates were observed using a photonic microscope (VWR®) to determine the shape, clustering mode and mobility of the cells. The isolates were then tested for their growth at different temperatures (10°C, 15°C and 45°C), pH (2, 4 and 9.6) and NaCl concentrations (4% and 6.5%) and for their type of fermentation using the semi-solid medium of Gibson and Abdel-el-Malek (Guiraud, 1998).

Screening of the LAB isolates for antimicrobial activity against pathogenic strains

The antimicrobial activity of the LAB isolates was tested toward 16 pathogenic strains available in our laboratory (Table 1). These microorganisms are composed of 12 strains of bacteria, two strains of yeasts and two strains of moulds. Bacterial strains, yeasts and moulds were stored at -80°C respectively in BHI broth, Yeast Glucose Peptone broth and Malt Extract, supplemented with 20% glycerol (v/v).

Antibacterial activity was evaluated by spot method (Fleming *et al.*, 1975). An aliquot of 2.5 µL spot of an 18-h culture of LAB isolate was deposited on modified MRS agar [0.2% (w/v) glucose, 1.2% (w/v) agar] in order to reduce acid production, minimize hydrogen

peroxide and acetic acid formation (Schillinger and Lucke, 1989). The spot was duplicated and the MRS broth without culture was used as negative control. Petri dishes containing spots were incubated anaerobically at 37°C for 24 h to allow the growth of LAB colonies. A volum of 0.1 mL of the 18-h culture of each indicator strain was used to inoculate 7 mL of a semi-aggregated Brain Heart Infusion (BHI) medium [0.7% (w/v) agar] maintained at 45°C, and pourred over the modified MRS petri dishes where the tested isolates grew. The plates were incubated again at 37°C aerobically to allow the indicator strains to develop. The size of the inhibition zones was measured after 24 and 48 h of incubation. Inhibition is assessed positive if the diameter of the clear zone around the colonies of the isolate tested is greater than or equal to 2 mm. The clear area around the colonies was measured and the results were reported in mm.

Antifungal activities were determined using superposition method as described by Magnusson *et al.* (2003). The LAB isolates were streaked in two lines of approximately 2 cm on MRS agar (Liofilchem, Italy) and the Petri dishes were incubated at 37°C for 48 h under anaerobic conditions. Afterwards, holes were overlaped with 10 mL of sweet malt extract (0.7% agar) containing 0.1 mL of mushroom spore suspension (10^5 spores mL^{-1}) and incubated at 30°C for 48 h. The inhibition zones were measured and the degree of inhibition was calculated as the area of inhibited growth in relation to the total area of the Petri dish. The scale was the following: - : no visible inhibition; *: no fungal growth on 0.1-3 % of plate area; **: no fungal growth on 3-8 % of plate area; ***: no fungal growth on >8 % of plate area. 8% of the surface of the Petri dish corresponds to an inhibition zone of approximately 12.73 mm in diameter.

Effects of pH-adjusted and catalase on antibacterial activity

Tested isolates with an inhibition zone greater than or equal to 12 mm of diameter were selected to evaluate effects of pH-adjusted and catalase on antibacterial activity using well diffusion test with a cell-free supernatant (Tagg and McGiven, 1971). Cultures were centrifuged at 8,000g at 4°C for 10 min. The supernatant was filtered through a 0.2 µm sterile millipore syringe filter to obtain a cell-free filtrate. For agar diffusion method, 15 mL of BHI soft agar (0.7%) maintained at 45°C were mixted with 0.1 mL of each indicator bacteria (approximately 10^7 CFU mL^{-1}) and poured into a petri dish. After solidification, three wells (6 mm diameter) were drilled and 35 µL of acellular supernatant was introduced into the first well; the remaining acellular supernatant was adjusted to pH 6.00 with 1 $mol.l^{-1}$NaOH, to exclude inhibitory activity due to organic acids. The sample was filtered and 35 µL was introduced into the second well. The remaining neutralized acellular supernatant was then treated with 1 mg mL^{-1} catalase at 25°C for 30 min to hydrolyze hydrogen peroxide. The resulting supernatant was filtered and 35 µL was introduced into the third well. The petri dishes were aerobically incubated at 37°C for 48 h. The inhibition zones were measured and expressed in mm at 24 and 48 h. When inhibition zones are found in the third well, the isolates were considered suitable for producing BLS.

Pattern of carbohydrate utilization and presumptive identification of LAB isolates

LAB isolates exibiting antimicrobial activity were presumptively identified based on carbohydrates fermentation profile with API 50 CHL galleries according to the supplier's operating procedure (Biomerieux, France).

Genotypic identification by 16S rDNA sequencing

Genotypic identification was performed at the Genoscreen Laboratories in France. The genomic DNAs of the isolates were extracted using the NucléoSpin® Tissue (Macherey-Nagel, Germany) extraction kit according to the supllier's protocols (New England Biolab, USA). Then PCR amplification of two 16S ribosomal DNA fragments (rDNA) from the hypervariable regions V1-V2-V3-V4-V5 was performed per isolate and forward/reverse sequencing of the two amplified fragments (sequences 1 and 2) was performed. Once the sequencing was completed, the sequences were

assembled using the Sequencher software (Version 4.7) marketed by Gene Codes Corporation. The contigs were then compared to the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov). Database of 16S ribosomal RNA sequences (Bacteria and Archaea) were compared and the BLAST program « Somewhat similar sequences (blastn) » was performed. Nucleotide sequences obtained in the present study have been assigned the GenBank Accession Nos.MN241996 to MN242007 (Table 5).

Evolutionary analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-4838.47) is shown.

The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 31 nucleotide sequences. There were a total of 1567 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

Differentiation of related species by metabolic criteria

There is a great similarity between the 16s rDNA sequences of several LAB species. Thus among our isolates six are identified as *Lb. plantarum/pentosus, one as Lb. plantarum/pentosus/paraplantarum* and one as *Lb. oris/fermentum*. Phenotypic characteristics such as the carbohydrates metabolism may distinguish isolates of *Lb. plantarum* from those of *Lb. pentosus* and from those *of Lb. paraplantarum,* and isolates of *Lb. oris* from those of *Lb. fermentum*. Indeed, *Lb. pentosus* strains fermented glycerol and D-xylose and did not ferment melizitose while *Lb. plantarum* fermented it and did not ferment glycerol and D-xylose (Tailliez *et al.*, 1996). Although *Lb. plantarum, Lb. pentosus and Lb. paraplantarum* are genetically related species with several common characteristics, a few phenotypic criteria can be used to differentiate them. The species *Lb. paraplantarum*can be identified by their inability to use α-methyl-D-mannoside (Pot *et al.*, 2014).

Strains of *Lb. oris* differ from those of *Lb. fermentum* in their ability to ferment amygdalin, D-arabitol and β-gentiobiose (Farrow and Collins, 1988). These metabolic criteria were used for identification at the species level.

Statistical analyses

All experiments were conducted at least in triplicate. Statistical analyses of the data were performed using the MINITAB 18.1. A simple statistical analysis was used to get means and standard deviations for the inhibition zone diameter.

Results

From preliminary tests (Gram, catalase, sporulation and organic acid production), 97 isolates of Gram-positive, catalase-negative, non-spore-forming and organic acid-producing bacteria were selected for screening of antimicrobial activity.

Antimicrobial activity

Out of 97 isolates, 17 presumptive LAB showed antimicrobial properties with inhibition zones of 12 mm of diameter or greater against at least one of the pathogenic tested strain (Table 2).

Some of the isolates showed inhibitory activity against a single or several indicator strains. The growth of pathogenic strains *Salmonella infantis, Bacillus cereus, Salmonella typhimurium, Listeria monocytogenes, Pseudomonas aeruginosa, Echerichia coli, Shigella dysenteriae* was inhibited by 47.06%; 29.41%; 23.53%; 23.53%; 17.65%;11.76%; 11.76% of the 17 LAB isolates respectively. The diameters of inhibition zones varied from 2 to 22mm.

Table 1. Indicator strains tested in the study.

Indicator strains	Media*/ Tempera-ture	Origin, references
Gram positive Bacteria		
Bacillus cereus NVH391-98	BHI/37°C	Food intoxication, furnished by INRA, France [a]
Bacillus cereus LMG13569	BHI/37°C	Culture collection of the Metropolitan University of London
Listeria monocytogenes NCTC 9863	BHI/37°C	
Staphylococcus aureus ATCC 2523	BHI/37°C	Culture collection of Département Technologie Alimentaire de l'IRSAT, Burkina Faso
Staphylococcus epidermidiss	BHI/37°C	
Gram negativeBacteria		
Yersinia enterocolitica BT3ST5 27	BHI/37°C	Culture collection of the Metropolitan University of London
Salmonella enteridis P167807	BHI/37°C	
Salmonella infantis SKN 557	BHI/37°C	Culture collection of the University of Copenhagen
Escherichia coli 81 nr.149 SKN 541	BHI/37°C	
Salmonella typhimurium O : 1036340P/t49	BHI/37°C	Culture collection of the Metropolitan University of London
Shigella dysenteriae 370	BHI/37°C	
Pseudomonas aeruginosa ATCC	BHI/37°C	Culture collection of Département Technologie Alimentaire de l'IRSAT, Burkina Faso
Yeasts		
Candida tropicalis	YGP/25°C.	*Fura*[b]
Candida albicans	YGP/25°C.	Culture collection of Département Technologie Alimentaire de l'IRSAT, Burkina Faso
Moulds		
Aspergillus fumugatus,	PDA/25°C	Maïze[c]
Aspergillus flavus	PDA/25°C	Peanut[c]

[a]Lund *et al.*, 2000; [b]Lindegaard-Pedersen *et al.*, 2012 [c]Compaoré *et al.*, 2017, *BHI : BrainHeart Infusion ; YGP : Yeast Glucose Peptone ; PDA : Potato Dextrose Agar.

The growth of *Salmonella enteridis, and Staphylococcus aureus,* was inhibited separately by 29.41% of isolates with inhibition zones ranging from 2 to 11mm in diameter. Of the 12 indicators bacteria, three pathogenic strains *Bacillus cereus* NVH391-98, *Yersinia enterocolitica* BT3ST 527 and *Staphylococcus epidermidis* resisted to the inhibitory action of the 17 LAB isolates tested.

The antifungal activity of the isolates was tested against *Aspergillus flavus*, *Aspergillus fumugatus*, *Candida albicans and Candida tropicalis*. Of the 17 isolates retained, 14 isolates (82.35%) inhibited both the growth of *Aspergillus flavus* and *Aspergillus fumugatus*. The largest inibition zones superior at 8% of petri dish surface were obtained with *Aspergillus flavus* (Table 2). However, none of the LAB isolates had an inhibitory action against *Candida albicans* and *Candida tropicalis* (results not shown).

For the effects of pH-adjusted and catalase on antibacterial activity,the 17 LAB isolates expressing antimicrobial activity were tested.Thus, the cell-free supernatant of five LAB isolates(TA2c, TA2j,TB4c, TB5a and TE5a) were tested against *Salmonella infantis* SKN 557 and it observed that two isolates (TA2c and TB4c) maintained their inhibitory action after neutralization of the action of acidity as well as after the treatment with catalase to eliminate action of hydrogen peroxide, with inhibition zones diameters ranging from 12.33 to 16.17 mm (Table 3a).The cell-free supernatant of the three isolates (TA2j, TB5a and TE5a) showed no inhibitory activity against *Salmonella infantis* SKN 557.

Table 2. Antimicrobial activity spectrum (by spot method) of LAB isolated from tomato pulp.

LAB Isolates	Indicators strains													
	L. monocytogenes NCTC 9863	*Ps. aeruginosa ATCC*	*Staph. aureus* ATCC 2523	*E. coli nr.149 541.*	*81 S. infan-tis* SKN 557	*S. typhimurium* O :10363 40P/t49	*Sh. dysen-teriae 370*	*S. enterid is P16780 7.*	*B. cereus LMG135 69*	*B. cereus NVH391-98*	*Staph. epidermidis*	*Y. enterocolitica BT3ST5 27*	*A. flavus*	*A. fumugatus.*
TA1e	-	-	-	-	-	-	+++	-	-	-	-	-	***	*
TA1j	+++	-	-	-	+++	-	+	-	-	-	-	-	-	-
TA2c	+	-	-	-	+++	-	-	-	-	-	-	-	-	-
TA2j	+++	-	-	-	+++	-	-	-	-	-	-	-	-	-
TB3c	-	-	-	-	-	+	-	-	+++	-	-	-	***	***
TB4c	-	-	-	-	+++	-	-	-	-	-	-	-	***	***
TB5a	-	-	-	-	+++	-	-	-	-	-	-	-	***	***
TD1h	-	-	-	-	-	-	-	-	++	-	-	-	***	**
TD3i	-	+++	-	-	-	-	-	-	-	-	-	-	***	***
TE2e	-	-	++	-	-	+	-	-	++	-	-	-	***	**
TE2i	-	-	++	-	-	-	-	-	+++	-	-	-	***	**
TE4j	-	+	-	-	-	-	-	+	++	-	-	-	**	***
TE5a	-	-	++	-	+++	-	-	+	-	-	-	-	*	**
TE5g	-	-	+	+++	+++	-	-	-	-	-	-	-	*	*
TF1j	-	-	++	+	+++	-	-	++	-	-	-	-	**	***
TF4b	-	-	-	-	-	+++	-	++	-	-	-	-	***	**
TF5c	+	++	-	-	-	+++	-	+	-	-	-	-	***	***
% of isolates	23.53	17.65	29.41	11.76	47.06	23.53	11.76	29.41	29.41	0	0	0	82.35	82.35

- : no inhibition; + : 2 ≤ Inhibition zone (IZ) ≤ 7mm; ++ : 8mm ≤ IZ ≤ 11mm; +++: IZ≥ 12mm. ; * : no fungal growth on 0.1-3% of petri dish surface ** : no fungal growth on 3-8% of petri dish surface ; *** : no fungal growth on a surface > 8% of petri dish surface ; *Staph : Staphylococcus ; Sh. : Shigella, Ps : Pseudomonas ; E : Echerichia ; S : Salmonella ;B : Bacillus ; A : Aspergillus; L: Listeria; Y: Yersinia*.

The cell-free supernatants of the isolate TD3i tested against *Pseudomonas aeruginosa* ATCC, has also maintained its activity after the treatments, with inhibition zones diameters ranging from 20.67 to 23.33 mm (Table 3a). The supernatants of three isolates (TA1e, TA1j and TE2i) showed no inhibitory activity against their target pathogen strain after the treatments (Table 3b) and the supernatants of the other 8 isolates (TB3c, TD1h, TE4j, TE2e, TE5g, TF1j, TF4b and TF5c) showed no inhibitory activity against their target pathogen strain before and after traitements(results not shown).The cell-free supernatants of tree isolates (TA2c, TB4c, TD3i) have maintained their antagonistic activities after the pH adjustment and the traitement with catalase and were then capable of producing bacteriocine like subtances (BLS) against *Salmonella infantis* SKN 557 (isolatesTA2c and TB4c) and toward *Pseudomonas aeruginosa* ATCC (isolate TD3i).

Phenotypic characteristics and presumptive identities of LAB isolates

The results of API 50 CHL galleries based on carbohydrates fermentation profiles of the 17 isolates showed that they belong to the genus *Lactobacillus and Pediococcus (Ped),* with a probability of identification ranging from 77.8 to 100% (Table 4).

The presumptive species include 11 isolates of *Lb. plantarum* (64.70%), three isolates of *Lb. fermentum* (17.65%), one isolate of *Lb. pentosus* (5.88%) and two isolates of *Ped. acidilactici* (11.76%).

Lb. plantarum isolates have rod-shaped cells and are all homofermentative. They were able to grow at 15°C, 45°C, pH 4, pH 9.6, at 4% and 6.5% NaCl. Most of the isolates (90.90%) was able to grow at 10°C. None of these isolates was able to grow at pH 2.

Table 3a. Antibacterial activity (inhibition zone in mm by well diffusion method) of acellular supernatants, pH adjusted supernatants and pH adjusted supernatants treated with catalase of LAB isolated from tomato fresh pulp, against *Salmonella infantis* SKN 557 and *Pseudomonas aeruginosa* ATCC.

Isolates of LAB	Diameters of the inhibition zones in mm						S pH
	Salmonallainfantis			*Pseudomonas aeruginosa*			
	S	AS	AS + C	S	AS	AS + C	
TA2c	12.33 ± 0.58	13.33 ± 0.58	12.5 ± 0.50	-	-	-	4.64
TB4c	12.67± 0.58	15.50± 0.50	16.17± 0.29	-	-	-	4.06
TD3i	-	-	-	20.83 ± 0.76	23.33 ± 0.58	20.67 ± 0.58	4.15

S : supernatant ; AS : pH-Adjusted ssupernatant ; AS+C : pH-Adjusted supernatant in presence of catalase ; - : not determined.

The isolate related to *Lb. pentosus* has rod-shaped cells and is heterofermentative with gas production from glucose. This isolate was able to grow at 15°C, 45°C, pH 4, pH 9.6, and in the presence of 4% and 6.5% NaCl, but could not grow at 10°C and pH 2.

Lb.fermentum isolates have rod-shaped cells and are all heterofermentative with gas production from glucose. They were able to grow at 10°C, 15°C, 45°C, pH 4, pH 9.6 and in the presence of 4% NaCl, but could not grow at pH 2 and in the presence of 6.5% NaCl. *Ped.acidilactici* isolates have shell-shaped cells and are homofermentative. They were able to grow at 10°C, 15°C, 45°C, pH 4, pH 9.6 and in the presence of 4% and 6.5%NaCl, but could not grow at pH 2.

Table 3b. Antibacterial activity (inhibition zone in mm by well diffusion method) of acellular supernatants, pH adjusted supernatants and pH adjusted supernatants treated with catalase of LAB isolated from tomato fresh pulp, against *Bacillus cereus LMG13569*, *Staphylococcus aureus* ATCC 2523 and *Shigelladysenteriae* 370.

Isolates of LAB	Diameters of the inhibition zones in mm									S pH
	Listeria monocytogenese			*Bacillus cereus*			*Shigella dysenteriae*			
	S	AS	AS + C	S	AS	AS + C	S	AS	AS + C	
TA1e	-	-	-	-	-	-	6.17 ± 0.29	0	0	4.17
TA1j	7.83 ± 0.29	0	0	-	-	-	-	-	-	4.59
TE2i	-	-	-	8 ± 0.00	0	0	-	-	-	4.18

S: supernatant ; AS : pH-Adjusted supernatant ; AS+C : pH-Adjusted supernatant in presence of catalase ; - : not determined.

Genotypic identification and evolutionary analysis

Presumptive identification by carbohydrates fermentation profile and biochemical criteria of the isolates made it possible to group them and to select 12 representative isolates for genotypic characterization and formal identification by 16S rDNA sequencing. The identification of isolates on BLAST nucleotide to nucleotide at NCBI based on their partial 16S rDNA sequences showed 99.8% to 100% similarity with related reference strains (Table 5). From the 12 isolates, six isolates (TB3c, TD1h, TE2i, TF1j, TF4b and TE4j) were identified as *Lb. plantarum/pentosus*, two isolates (TA1j and TA2j) were identified as *Lb. fermentum,* one isolate (TA1e) was identified as *Lb. plantarum*, one isolate (TB5a) was identified as *Lb. plantarum/paraplantarum/pentosus*, one isolate (TA2c) was identified as *Lb.fermentum/oris and* one isolate (TE5a) was identified as *Ped. acidilactici*.

A dendogram was constructed from the DNA-DNA similarities of our isolates with reference strains obtained from NCBI databases (Fig. 1). The isolates TA1e, TB5a, TF1j, TF4b and TE4j identified as *Lb. plantarum* and the isolate TE2i identified as *Lb. pentosus/plantarum* have grouped with the reference

strains *Lb. plantarum* (GenBank Accession : MH016559.1CP037429.1,CP025690.1,MK530235.1, CP025285.1) and *Lb. pentosus* (GenBank Accession : KY777710.1, KJ802480.1, KF668473.1) with 100% smilarity. Isolates TA1j and TA2j identified as *Lb. fermentum* and isolate TA2c identified as *Lb. fermentum/oris* grouped with the reference strains *Lb. fermentum* (GenBank Accession: MK246000.1, MK243452.1, MK235130.1, MK235129.1, MK033872.1) with 96% similarity. The isolate TE5a identified as *Ped.acidilactici* formed a group with a reference strain of *Ped. acidilactici CP035154.1* with a similarity of 99%. However, two isolate TB3c and TD1h identified as *Lb. plantarum* formed a group with a similarity of 70% independently of the reference strains of *Lb. plantarum*.

Table 4. Phenotypic characteristics of 17 LAB isolates collected from tomato fresh pulp.

Isolates code	Shape	CO_2	T(°C)			pH			[NaCl] (%)		Species	% ID API 50 CHL
			10	15	45	2	4	9.6	4	6.5		
TA1e	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	99.7
TA1j	Rods	+++	+	+	+	-	+	+	+	-	*Lb. fermentum*	97.2
TA2c	Rods	+++	+	+	+	-	+	+	+	-	*Lb. fermentum*	96.2
TA2j	Rods	+	+	+	+	-	+	+	+	-	*Lb. fermentum*	97.7
TB3c	Rods	++	-	+	+	-	+	+	+	+	*Lb. pentosus*	99.7
TB4c	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	100
TB5a	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	100
TD1h	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	99.8
TD3i	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	100
TE2e	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	99.5
TE2i	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	99,5
TE4j	Rods	-	-	+	+	-	+	+	+	+	*Lb. plantarum*	100
TE5a	cocci	-	+	+	+	-	+	+	+	+	*Ped. acidilactici*	77.8
TE5g	cocci	-	+	+	+	-	+	+	+	+	*Ped. acidilactici*	77.8
TF1j	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	100
TF4b	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	100
TF5c	Rods	-	+	+	+	-	+	+	+	+	*Lb. plantarum*	100

- : no growth or no gas production, +: growth or low gas production, ++: Medium gas production, +++: high gas production.

Differenciation of isolates

The metabolism of carbohydrates has made it possible to distinguish isolates of *Lb. plantarum* from those of *Lb. pentosus* as well as from those of *Lb. paraplantarum*. Four of the six isolates identified as *Lb. plantarum/pentosus* could not ferment glycerol and D-xylose but they were able to ferment melizitose. These results lead to conclude that the four isolates (TD1h, TF1j, TF4b and TE4j) belongs to the species *Lb. plantarum*. The isolateTB3c was able to ferment glycerol and D-xylose but could not ferment melizitose, so it belongs to the species *Lb. pentosus*. However, the method did not allow to conclude at the species level for the isolate TE2i because it fermented both D-xylose and melizitose. The isolate TB5a identified as Lb. *plantarum/paraplantarum/pentosus* was able to ferment α-methyl-D-mannopyranoside, therefore it does not belong to the *Lb.paraplantarum* species. Also, the isolate TB5a fermented melizitose and was unable to ferment glycerol and D-xylose, and could be identified as *Lb. plantarum*. As for the isolate TA2c identified as *Lb. oris/fermentum,* it could not ferment amygdalin, D-arabitol and β-gentiobiose and therefore it does not belong to the species *Lb. oris* but it belongs to the species *Lb. fermentum*. Ultimately, the 12 isolates are composed of *Lb. plantarum* (6 isolates), *Lb. plantarum/pentosus* (1 isolate) *Lb.*

pentosus (1 isolate), *Lb. fermentum* (3 isolates) and *Ped. acidilactici* (1 isolate).

Pattern of carbohydrate utilization of LAB

All the 12 LAB isolates are able to ferment galactose, D-glucose and D-fructose. In addition to the three carbohydrates, the isolates identified as *Lb. fermemtum* fermented ribose, maltose, melibiose, saccharose, D-raffinose, gluconate and 5 ketogluconate; 66% of them fermented D-xylose, D-mannose, lactose, trehalose and 33% of them fermented L-arabinose, esculin and cellobiose.

The *Lb. pentosus* isolate fermented glycerol, ribose, D-xylose, D-mannose, rhamnose, mannitol, sorbitol, α-methyl-D-glucopyranoside, N-acetyl glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, D-raffinose, β-gentiobiose, D-turanose, D-arabitol and gluconate (Table 6).

Table 5. Genotypic identification of 12 LAB isolates collected from tomato fresh pulp.

Isolates Code	Presumptive identification (API 50CHL)	Genotypic identification (rDNA 16S)		GenBankacces-sionnumber	Final identities
		Idenities	SequenceSimilarity (%)		
TA1e	*Lb. plantarum*	*Lb. plantarum*	100	MN241997	*Lb. plantarum*
TD1h		*Lb. plantarum/pentosus*	99.8/99.8	MN242003	*Lb. plantarum*
TE2i		*Lb. plantarum/pentosus*	99.9/99.9	MN242004	*Lb. plantarum/pentosus*
TF1j		*Lb. plantarum/pentosus*	100/100	MN242006	*Lb. plantarum*
TF4b		*Lb. plantarum/pentosus*	99.9/99.9	MN242007	*Lb. plantarum*
TE4j		*Lb. plantarum/pentosus*	99.9/99.9	MN241996	*Lb. plantarum*
TB5a		*Lb. plantarum/paraplantarum/pentosus*	100/100/100	MN242002	*Lb. plantarum*
TB3c	*Lb. pentosus*	*Lb. plantarum/pentosus*	100/100	MN242001	*Lb. pentosus*
TA1j	*Lb. fermentum*	*Lb. fermentum*	100	MN241998	*Lb. fermentum*
TA2c		*Lb. fermentum/oris*	100/100	MN241999	*Lb. fermentum*
TA2j		*Lb. fermentum*	100	MN242000	*Lb. fermentum*
TE5a	*Ped. acidilactici*	*Ped. acidilactici*	100	MN242005	*Ped. acidilactici*

The *Lb. plantarum* isolates fermented the same carbohydrates as those fermented by *Lb. pentosus* except glycerol, D-xylose and α-methyl-D-glucopyranoside. In addition to these carbohydrates, *Lb. plantarum* isolates fermented L-arabinose, α-methyl-D-mannopyranoside and melezitose.

The *Lb. plantarum/pentosus* isolate fermented also the same as those fermented by *Lb. pentosus* except glycerol. Furhermore, it fermented three carbohydrates such as L-arabinose, α-methyl-D-mannopyranoside and melezitose (Table 6). As for the *Ped.acidilactici* strain, it fermented L-arabinose, ribose, D-xylose, β-galactose, D-glucose, D-fructose, D-mannose, rhamnose, N-acetyl glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, trehalose, β-gentiobiose and D-tagatose (Table 6).

Discussion

Antibacterial activity of LAB species isolated from tomato fresh pulp

The present investigation revealed the presence of LAB in healthy fresh tomato pulp sampled from Ouagadougou in Burkina Faso, after enrichment and isolation. Screening of our LAB isolates for the antimicrobial activity showed some of the isolates (17.5%) belonging to the species *Lb. plantarum, Lb. fermentun, Lb. plantarum/pentosus, Lb. pentosus and Ped. acidilactici* exhibited antimicrobial activities against pathogenic and indesirable microorganisms. Our result is comparable to that of Biswal *et al.* (2012) who obtained a rate of 13.09% of isolates with antimicrobial activity when they screened the LAB isolated from traditional fermented products from India for antimicrobial activity.

Table 6. Carbohydrates fermentation profile of the 12 LAB isolates collected from tomato fresh pulp.

N°	Carbohydrates	Isolates of LAB from tomato				
		Lb. fermentum (n=3)	*Lb. pentosus* (n= 1)	*Lb. plantarum* (n= 6)	*Lb. plantarum /pentosus* (n=1)	*Ped. acidilactici* (n=1)
1	Glycerol	0	100	0	0	0
2	Erythritol	0	0	0	0	0
3	D-Arabinose	0	0	0	0	0
4	L-Arabinose	33	0	100	100	100
5	Ribose	100	100	83	100	100
6	D-Xylose	66	100	0	100	100
7	L-Xylose	0	0	0	0	0
8	Adonitol	0	0	0	0	0
9	β-Methyl–Dxyloside	0	0	0	0	0
10	Galactose	100	100	100	100	100
11	D-Glucose	100	100	100	100	100
12	D-Fructose	100	100	100	100	100
13	D-Mannose	66	100	100	100	100
14	L-Sorbose	0	0	0	0	0
15	Rhamnose	0	100	50	100	100
16	Dulcitol	0	0	0	0	0
17	Inositol	0	0	0	0	0
18	Mannitol	0	100	100	100	0
19	Sorbitol	0	100	100	100	0
20	α-Methyl-D-mannoside	0	0	66	100	0
21	α-Methyl-D-glucoside	0	100	0	100	0
22	Nacetyl glucosamine	0	100	100	100	100
23	Amygdalin	0	100	100	100	100
24	Arbutin	0	100	100	100	100
25	Esculin	33	100	83	100	100
26	Salicin	0	100	100	100	100
27	Cellobiose	33	100	100	100	100
28	Maltose	100	100	100	100	0
29	Lactose	66	100	100	100	0
30	Melibiose	100	100	100	100	0
31	Saccharose	100	100	100	100	0
32	Trehalose	66	100	83	100	100
33	Inulin	0	0	0	0	0
34	Melezitose	0	0	100	100	0
35	D-Raffinose	100	100	100	100	0
36	Starch	0	0	0	0	0
37	Glycogen	0	0	0	0	0
38	Xylitol	0	0	0	0	0
39	β Gentiobiose	0	100	83	100	100
40	D-Turanose	0	100	83	100	0
41	D-Lyxose	0	0	0	0	0
42	D-Tagatose	0	0	0	0	100
43	D-Fucose	0	0	0	0	0
44	L-Fucose	0	0	0	0	0
45	D-Arabitol	0	100	33	100	0
46	L-Arabitol	0	0	0	0	0
47	Gluconate	100	100	83	100	0
48	2 keto gluconate	0	0	0	0	0
49	5 keto gluconate	100	0	0	0	0

n = number of isolates.

Our LAB isolates displayed antibacterial activities against gram positive pathogenic bacteria (*L. monocytogenes, Staph. Aureus* and *B. cereus)* and gram negative pathogenic bacteria *(Ps. aeruginosa, S. infantis, S. typhimurium, S. enteridis, E. coli* and *Sh. dysenteriae*). Previous studies have also reported antimicrobial activity of *Lb. plantarum, Lb. fermentum, Lb. pentosus* and *Ped. acidilactici* isolated from fruits and vegetables that had the ability to inhibit pathogenic strains such as *Ps. aeruginosa, S. typhi, Salmonella sp., Staph. aureus, E. coli* (*Chiu et al.*, 2007, Tamang *et al.*, 2009; Vitali *et al.*, 2012). LAB can produce antimicrobial substances such as sugar catabolites (lactic acid and acetic acid); oxygen catabolites (hydrogen peroxide); proteinaceous compounds (bacteriocins, other low-molecular-mass peptides, and antifungal peptides/proteins); fat and amino acid metabolites (fatty acids, phenyllacticacid, and OH-phenyllactic acid); and others such as reuterin and reutericyclin which are capable to inhibit the growth of pathogenic and spoilage microorganisms (Makras *et al.*, 2006).

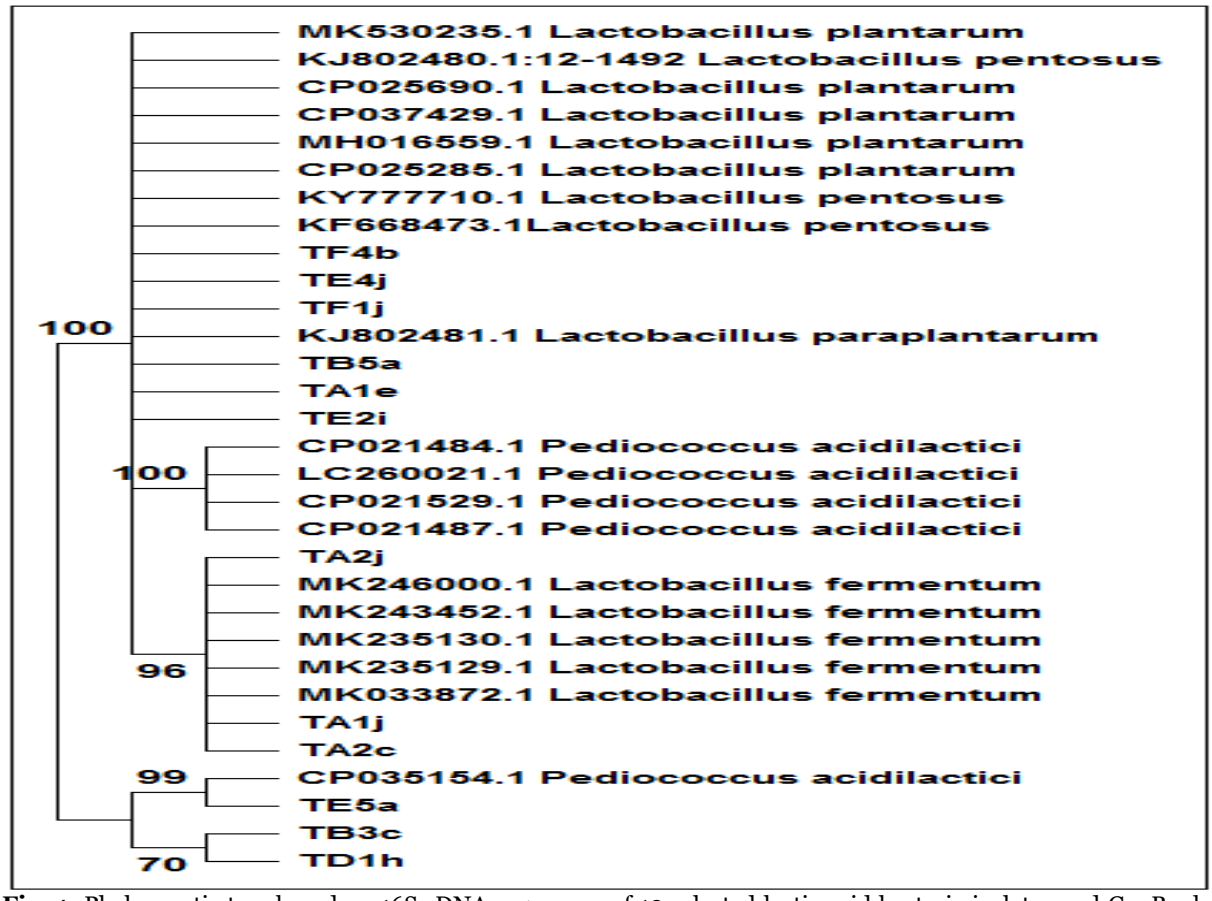

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of 12 selected lactic acid bacteria isolates and GenBank related reference strains (*Lactobacillus plantarum, Lactobacillus paraplantarum, Lactobacillus pentosus and Pediococcus acidilactici*).

In addition, inhibitory activity was not affected by the effect of acid neutralization or treatment with catalase for three LAB free-cell supernatants, indicating that the growth inhibition of the pathogenic bacteria *S. Infantis and Ps. aeruginosa* was not due to the production of organic acids or hydrogen peroxide. These isolates belonging to species *Lb. fermentum and Lb. plantarum* are considered suitable for the production of a bacteriocin-like substance. Studies also showed that strains *of Lb. fermentum and Lb. plantarum* produce bacteriocins, some of which inhibited both the growth of gram-positive and gram-negative bacteria (Savadogo *et al.,* 2004; Rizzello *et al.,* 2014). The loss of their antibacterial activity after neutralization of the action of acidity and in the presence of catalase for the other three isolates, prove

that their antimicrobial activity was due to organic acids. However 11 isolates showed inhibitory activity by the spot method and were characterized by a lack of inhibitory activity with the well method, meaning that their inhibiting substances are not extra-cellular.

Antifungal activity of LAB species isolated from tomato fresh pulp

For the antifungal activity, our 14 isolates belonging to the species *Lb. plantarum*, *Lb. pentosus* and *Ped. acidilactici* showed inhibitory activity against both *A. flavus* and *A. fumugatus* which are micotoxigenic fungi. The production of antifungal compounds by lactobacilli, mainly strains of *Lb. plantarum*, was reported by Ström *et al.* (2002). The use of these isolates as starter cultures could insure the safety of the fermented food products because of their antagonistic actions toward bacteria and fungi. Indeed, synthesis of antimicrobial compounds is one of the main criteria to select LAB starter cultures for food fermentation (Di cagno *et al.,* 2013). The absence of inhibitory activity of our isolates against yeast species (*C. albicans* and *C. tropicalis*) could be explained by the fact that yeast species form generally a stable symbiosis with LAB, particularly lactobacilli (Sawadogo-Lingani *et al.,* 2008). However, Garmasheva *et al.* (2016) repported the antagonistic activity of some isolates of *Lactobacillus against C. albicans*.

Phenotypical and genotypical characteristics of LAB species with antimicrobial activities found in fresh pulp of tomatoes

Biochemical analysis showed that all our *Lb. plantarum* isolates were able to grow at 45°C and almost all our *Lb. fermentum* isolates were able to grow at 15°C and also at 10°C. Similar results have also been reported by Samadoulougou-Kafando *et al.* (unpublished results) when they characterized LAB isolated from fresh pulp of mango in Burkina.Faso. However, according to the growth temperature specifications, *Lb. plantarum* is mesophilic and cannot grow at 45°C (Bringel *et al.,* 2005); *Lb. fermentum* is not able to grow at 15°C (Hammes and Vogel, 1995). The atypical nature of our strains can be explained by the fact that a number of LAB have evolved to adapt to specific niches. These organisms have gained some specific genes and have lost other genes that are unnecessary (Endo and Dick, 2014). The genotypical identification by 16S rDNA sequencing has made it possible to identify some isolates at species level. For the isolates identified as *Lb. plantarum/pentosus*, it was previously demonstrated by DNA/DNA homology measurements that the species *Lb. plantarum* includes two species: *Lb. plantarum* and *Lb. pentosus* (Dellaglio *et al.,* 1975; Zanoni *et al.,* 1987). The metabolism of carcohydrates has made it possible to distinguish these isolates except the isolate TE2i (*Lb. platarum/pentosus*). Indeed, Tailliez *et al.* (1996) noted that the use of carbohydrates metabolism is not always effective because some strains of these two species may have atypical characteristics. The results from the carbohydrates fermentation profiles of 12 identified LAB isolates indicated that they fermented mainly pentoses, hexoses and disaccharides to produce organic acids. Our isolates showed strong fermentation ability and can be used for fermentation purposes. Isolates of *Lb. fermentum*, *Lb. plantarum*, *Lb. pentosus*, *Lb plantarum/pentosus* and *Ped.acidilactici* with their appropriate antimicrobial activities and carbohydrates fermentation profiles will be potential candidates to be used as starter cultures.

Conclusion

The research for lactic acid bacteria with antimicrobial activity in tomato pulp from Ouagadougou (Burkina Faso) showed that tomato harbour a diversity of LAB species. The identified species *Lb. plantarum, Lb. pentosus, Lb. fermentum* and *Ped. acidilactici* showed high antimicrobial activity and high carbohydrate fermentation capacity. The isolates belonging to genus *Lactobacillus* mainly *Lb. plantarum, Lb. pentosus, Lb. fermentum,* and *Pediococcus* mainly *Ped. acidilactici*, are species well known for their importance in food product fermentation. Their use in the manufacture of fermented products will inhibit the proliferation of pathogenic or spoilage microorganisms and thus, ensure proper preservation of the finished products.

This study is a contribution to update knowledge on lactic acid bacteria from tomato and provides a selection of isolates with functional properties that can be used as starter cultures for lactofermentation of fruits and vegetables based products.

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