Annals of Arid Zone 63(1): 9-21, 2024

Biochemical, Physiological and Technological Properties of Lactic Acid Bacteria Isolated and Characterized from Hard and Soft Wheat

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Received: September 22, 2023 Accepted: March 29, 2024

Abstract: Population of lactic acid bacteria (LAB) adhering on three samples of wheat grain, viz. Algerian durum wheat, Canadian durum wheat and French soft wheat and a sample of bread flour, collected from the Semoulerie Industrielle de la Mitidja was cultured and identified to determine their diversity. Fifty four lactic acid bacteria were isolated. They belonged to 20 species of eight genera (*Pediococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Weissella*, *Streptococcus*, *Leuconostoc* and *Aerococcus*). The *Enterococcus* genus represented 42% of the isolated strains, followed by *Streptococcus* (19%), *Lactococcus* (11%), *Lactobacillus* (9%) and *Pediococcus* (9%), *Leuconostoc* (4%) and *Weissella* (4%) and the *Aerococcus* (2%). Nineteen biochemical and physiological parameters of LAB population were estimated along with four technological properties were studied. Oxidase, mannase, urease, nitrate reductase and haemolysis were negative for all species. All the 54 strains tested showed acidifying capacity, proteolytic nature and lipase activity. All except *Enterococcus acidominimus* B8 and B14, *Streptococcus thermophilus* B13, both strains of *Lactococcus lactis* ssp. Cremoris, *Lactococcus lactis* ssp. Lactis, Leuconostoc *mesenteroides* subsp. mesenteroides did not possess lacithinase activity. *Leuconoctoc mesenteroides* subsp. mesenteroides was the most efficient acid producing strain. It was closely followed by *Lactobacillus fermentum and Leuconostoc mesenteroides* subsp. *dectranicum*. Close similarity in their biochemical and physiological characters showed that efficiency of any new species for industrial and household suitability for fermentation can be predicted on the basis of similarity in their characters with *Leuconoctoc mesenteroides* subsp. *mesenteroides*.

Key words: Durum wheat, soft wheat, flour, lactic acid bacteria.

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Citation
Guetarni, H., Elkloei, B. and Ayadi, A. 2024. Biochemical, physiological and technological properties of lactic acid bacteria isolated and characterized from hard and soft wheat. Annals of Arid Zone 63(1): 9-21:

doi: 10.59512/aaz.2024.63.1.2

https://epubs.icar.org.in/index.php/AAZ/article/view/142841

Lactic acid bacteria are the most important group of industrial microorganisms in food fermentation producing a variety of metabolites which improve taste and texture of fermented foods (Bouchibane *et al.*, 2023). Lactic acid bacteria (LAB) are gram-positive bacteria and use carbohydrates as the only or main carbon source (George *et al.*, 2018). They are generally cocci or rods, and have high tolerance to low pH. Although LAB include more than 60 genera, the

genera frequently used for food fermentation are *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, Streptococcus, *Enterococcus*, *W. eissella*, etc. (Mokoena, 2017). LAB are being used for production of artisanal sourdoughs for thousands of years and of late also in industrial fermenters (Ennadir *et al.,* 2014). But in spite of industrial development artisanal sourdough bread prepared by the fermentation of dough using wild LAB remains a dominating process.

Wheat is a prominent source of dough. There are many classes of wheat *viz*. hard (winter/spring), soft/red winter, hard/soft white and Durum which vary in their protein and gluten content. Hard wheat contains higher protein content and is used in breads and quick breads. Soft, lower protein wheats are used in cakes, pastries, cookies, crackers, flatbreads and Asian-style noodles. Depending on the region of the world, wheat may be eaten as bread that is flat or loaved, leavened or unleavened, or processed into pasta or couscous but of all these sourdoughs is a major way.

Cereals, including wheat, are naturally contaminated with molds, yeasts and bacteria. But sometimes their growth can lead to serious alterations in the nutritional value and organoleptic characteristics of the grains (taste, smell, appearance). They can also influence vigor and germinative power of the seeds and molds can sometimes form mycotoxins. Generally, the microbial flora form a very fragile balance and lactic acid bacteria (LAB) plays a very important role in preserving this balance of the microbial flora and stabilizing the final products of fermentation (Caplice, 1999; Guetarni *et al.,* 2012; Guetarni, 2017) and prevent the degradation of food products (Ennadir *et al.,* 2014).

Fermentation changes the organoleptic (appearance, smell, flavor) and nutritional, biochemical and microbiological composition and functionalities (Benakriche *et al*., 2017). Fermentation causes a significant physicochemical modification of wheat (Gourchala *et al*., 2014) and proteolysis which would give a functional protein profile. According to Wang *et al*. (2021), in food the Lactic acid bacteria can decompose the protein and produce a peptides or free amino acids. Lactic acid bacteria and rarely yeasts are the leaders in the fermentation of durum wheat in Matmor (Benakriche *et al.*, 2016). Indeed, *Hamoum* is obtained after several years of spontaneous fermentation which could expose it to microbial contamination. A new non-Matmor method is developed to considerably reduce fermentation time (Merabti, 2015). It is also dependent on spontaneous fermentation. The *Hamoum* microflora is predominated by LAB (Gourchala *et al*., 2014). Wheat seeds contain amylolytic enzymes which release maltodextrins, maltose and glucose. Their endogenous enzymatic activities play an important role in the degradation of starch, which is considered a source of fermentable sugars (Ganzle, 2014). The decrease in carbohydrate content may be due to their use as fermentable substrates by the wheat microflora during fermentation (Bushuk and Rasper, 2012). LAB are used to produce a fermented products (fermented cereal products or high-fiber ingredients, such as sourdough bread, fermented wheat bran and whole-meal flours) (Manini *et al*., 2016). Food safety can be improved using this bacteria that can decrease pH through the production of lactic acid (Salvucci *et al.,* 2016).

Therefore, during this study we focused on LAB naturally present in/on wheat grain and flour and assess their biochemical, physiological and metabolic properties after their isolation and purification to identify the most efficient ones.

Material and Methods

The study was carried out on Algerian durum wheat (B), Canadian durum wheat (B′) and French soft wheat (B″). Wheat samples were collected from large closed metal silos of Semoulerie Industrielle de Mitidja (SIM) located in the north of Algeria (http://mapearta.com/fr/17320458). The samples were kept in paper bags. Besides the three types of wheat, samples of bread flour (F) were also collected from Semoulerie Industrielle from the cylinder device, in bags. After collection, samples were visually examined and the impurities were removed and then sieved (1.9 mm for durum wheat and 2 mm for soft wheat).

Culturing lactic acid bacteria

Processing of grain samples: The grain samples were processed for fermentation parallelly in two sets one with and another without vinegar. To begin with in first set 600 g of wheat was

placed in a sterile bottle and then 15 ml of water and 15 ml of 95° vinegar was added to it. Vinegar was added to reduce the pH of the medium necessary for fermentation of wheat by the indigenous flora especially LAB. In another set instead of vinegar 30 ml distilled water was added. These mixtures were kept in dark colored sterile bottles. These bottles are placed in a damp, dark place for a period of 4 months (Kermiche, 2013).

After incubation period, in the set without acetic acid, 60g of wheat was weighed into a sterile bottle and 200 ml of sterile distilled water was added to it. These bottles were placed in the dark for 72 hours. In the other set incubated with acetic acid 100 ml of vinegar and 100 mL of sterile distilled water were mixed with 60 g grain and theses bottles were also placed in dark for 72 hours. Subsequently the moist grains were crushed in a mortar (Fig. 1).

Processing of flour samples: One g of the flour was weighed in a sterile glass capsule under sterile conditions and using a micropipette, 3 mL of sterile water was poured into it.

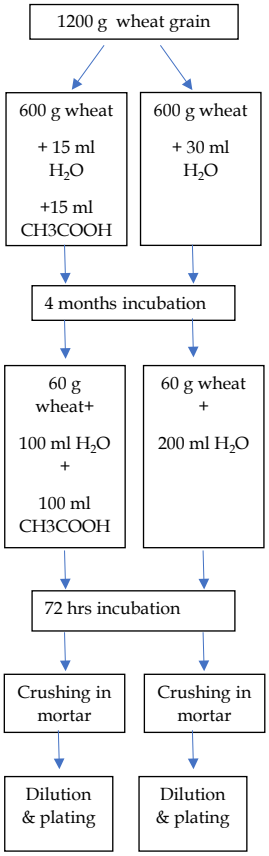

Fig 1. Preparation of grains samples for microbial culture

Then 1 mL of the crushed grain fluid was used to prepare the decimal dilutions by the dilution-plate technique. MRS medium was used for the growth of resident microorganisms. The Petri dishes were incubated at 37°C for 48 hours. Distinct colonies were removed aseptically and placed in MRS broth and incubated at 37°C for 24 hours. Several transplants were made to obtain a pure strain. Pure strains were maintained in MRS broth and subsequently identified (Larpent, 1997, Guetarni and Krarmouch, 2023).

Identification of isolated lactic acid bacteria

Macroscopic and microscopic examination

Gram staining was used to classify bacteria according to their Gram reaction, their morphology and their mode of association. The colonies obtained were observed under an optical microscope after fixing a smear and Gram staining (Guiraud, 2003).

Biochemical and physiological tests

All tests are carried out on young cultures prepared from preserved strains. A culture on MRS broth is prepared, from which a few colonies are taken using a sterile platinum loop and put into the tube containing the MRS broth, previously sterilized and incubated at 37°C for 24 to 48 hours. The biochemical and physiological parameters studied were:

Biochemical Characteristics

Catalase activity: A colony growing on MRS agar was put into a drop of hydrogen peroxide (H_2O_2) at 10 volumes. The appearance of bubbles revealing the release of oxygen indicated presence of catalase activity (Guetarni and Krarmouch, 2023).

Oxidase activity: A small area of filter paper was moistened with a few drops of Kovacs oxidase reagent and a small quantity of bacterial cells was spread on it. Oxidase-positive species give a purple color immediately or within 10 seconds (Savadogo and Traore, 2011, Guetarni and Krarmouch, 2023).

Nitrate reductase activity: This enzyme is capable of catalyzing the nitrate reduction reaction (NO_3^-). A culture in nitrate medium was inoculated and incubated at 37°C, nitrate reagent I (sulfanilic acid) was added followed by nitrate reagent II (α-naphthylamine).

Formation of red color indicated that the nitrate was degraded by the bacteria (Larpent, 1997).

Mannitol-mobility test: The test allows simultaneous research of the fermentation of mannitol and the study of the mobility of the strain. The medium was inoculated by central puncture in the pellet to the bottom of the tube and incubated for 24 hours at 37°C. The fermentation of mannitol is revealed by an acidification of the medium which causes the pH indicator to turn yellow. Mobility results in the diffusion of bacteria from the seeding line towards the periphery, creating turbidity. Immobile bacteria grow only along the central puncture (Agbankpe *et al.,* 2019).

Respiratory type: This test allows the classification of bacteria according to their respiratory types viz. facultive aero-anaerobic and aerobic (Benslimani, 2006). For this test, we used deep meat-liver (ML) agar distributed in pellet tubes. The medium was inoculated using a Pasteur pipette which is extended to the bottom of the tube and then raised in a spiral so as to uniformly inoculate the medium over the entire height. The medium was incubated at 37°C for 48 hours. After incubation, only the strict aerobic bacteria develop on the surface, the strict anaerobic bacteria develop at the bottom of the tube, the facultative aero-anaerobic bacteria develop over the entire height and micro-aerophilic bacteria develop in an intermediate zone of the agar.

Physiological characteristics

Growth in hostile environments: LAB were grown in a hypersaline MRS medium (pH 4 and 6.5 and NaCl 2%, 4% and 6.5%) at 37°C for 24 hours. The presence of cloudiness indicates growth (Belarbi, 2011).

Thermo-resistance: It was carried out by heating the liquid MRS medium seeded by the bacterial culture at a temperature of 65°C for 30 min (Teuber and Geis, 2006). It was then placed in the oven for 24 hours at 37°C. The presence of cloudiness indicates growth (Belarbi, 2011).

Resistance to tellurite: Tolerance to tellurite was tested by seeding in streaks, 0.4% potassium tellurite agar, by the cultures to be tested. After a period of 24 hours of incubation at 37°C, the resistant bacteria gave black colonies (Larpent, 1997).

Sherman milk culture: This test indicates the ability of bacteria to grow in the presence of methylene blue which is in a very oxidizing environment. Methylene blue is discolored by germs with reductase activity and can coagulate milk. A tube of milk with 1% methylene blue was inoculated and incubated at 37°C for 48 h to 72 h (Benazzouz, 2012).

Fermentative type: This test makes it possible to specify the type of fermentation metabolism carried out by the microorganism to transform the carbonaceous substrate and it consists of highlighting the formation of gas (CO_2). Young strains previously prepared were inoculated in tubes containing MRS broth, with a Durham bell. After incubation at 37°C for 24 to 48 hours, the presence or absence of gas in the bell indicated the fermentation type (Rhaiem *et al.,* 2016).

Urease test: The tubes containing urea indole were inoculated with lactic acid bacteria and incubated at 37°C for 18h to 24h, the positive result was interpreted by the change in color from orange to pink (Singleton, 1994).

Arginine dihydrolase (ADH), lysine decarboxylase (LDC) and ornithine decarboxylase (ODC) test: It is detected on Moeller medium, for each isolated strain seeded. These environments contain only one amino acid, the one for which we want to study covering the environment with sterile paraffin oil in order to create relative anaerobiosis. After 2 to 6 days of incubation at 37°C, the medium turns yellow due to the acidification of the medium by glucose fermentation (Carr *et al.,* 2002). The degradation of "arginine, lysine, ornithine" leading to the formation of ammonia is revealed by re-alkalization of the medium which returns to its initial color (purple) (Belarbi, 2011).

Acetoin production: Acetoin production is tested on Clark and Lubs medium (Guiraud, 2003) which is inoculated by the pure culture incubated at 37°C. After 24 hours and in a hemolysis tube, 0.5 ml of α-naphthol reagent at 6% in absolute alcohol (VP1) and 0.5 ml of a sodium hydroxide solution (NaOH) at 16% in distilled water (VP2) were added to ensure the Voges-Proskaeur reaction called the VP reaction. The tubes are carefully shaken and left in contact with the open air for 5 to 10 min at room temperature. The production of

acetoin resulted in the appearance of a red ring on the surface of the medium.

Culture on sunflower milk: The medium is prepared from sterile skimmed milk and added with sunflower tincture until a purple color is obtained, the pH is adjusted to 7.3. Seeding was carried out from a dense culture of the strain studied, followed by incubation at 37°C for 3 to 5 days. This environment allows several types of reaction to be observed: attack of lactose with acidification (coagulation of casein and turning red), attack of casein with alkalization (turning blue), peptonization of casein after or outside of any coagulation (lightening of the medium or degradation of the coagulant) and reduction of dye (discoloration) (Benazzouz, 2012).

Gelatin degradation test: Tubes of solidified gelatin (in pellet) were inoculated by central puncture in the pellet with the bacterial strains. These tubes were incubated at 37°C for 48 hours and then placed for approximately one hour at 4°C. Gelatin being a liquid protein at the incubation temperature, it is necessary to put the tubes in the cold before observing them (Delarras, 2007):

liquid medium (hydrolyzed gelatin : Gelatin +).

solid medium (non-hydrolyzed gelatin: Gelatin -).

Hemolysis test: Hemolysis was tested on blood agar medium added to 5 ml of fresh blood per vial. After having allowed the medium to cool, the sample is fresh on site and the 5 ml are added to the medium and mixed carefully then poured onto the box. After streaking our isolates, we incubate at 37°C for 24 hours to 48 hours. After incubation, the type of hemolysis was examined. It can be α hemolytic "partial hemolysis" (green color around the colonies), β-hemolytic "total hemplysis" (lightening around the colonies) or γ-hemolytic "non-hemolytic" (the medium is not modified) (Idoui *et al.,* 2009).

β-galactosidase: According to Delarras, 2007, the test is carried out by making a dense suspension of the bacteria tested in water at physiological pH and then using flamed and cooled forceps to add a disk impregnated with ONPG (Ortho-Nitro-Phenyl-β-D-Galactopyranoside). The tubes were put in the oven at 37°C for 24 hours. The presence of *β*-galactosidase results in the release of soluble yellow orthophenyl which appears after the incubation period.

Use of sugars on triple sugar iron (TSI) medium: The slope of the TSI medium is seeded by streaks and the base by central puncture followed by incubation at 37°C for 24 hours. The change of the base to yellow reflects the fermentation of glucose. The presence of gas bubbles means fermentation with gas production. The change in the slope to yellow reflects the use of lactose or sucrose or both at the same time. A black color means the production of hydrogen sulphide (H_2S) (Denis *et al.,* 2007).

Fermentation profile study: The study of the fermentation of the seven sugars was carried out in test tubes. BCP broth (9 mL) was added to the tubes to which 100 µl of each strain were added. Anaerobic conditions were ensured by adding a layer of paraffin oil to the surface and incubation was done at 37°C for 24 to 48 hours. The change to yellow in the pH indicator of the medium reflected the fermentation of the sugar tested (Gonzàlez *et al.,* 2007).

Technological aptitudes of isolated lactic acid bacteria

Acidifying power: Measuring the acidifying activity consists of monitoring, on the one hand, the pH evolution of the different cultures as a function of time and, on the other hand, simultaneously measuring the total acidity using sodium hydroxide. We start by preparing 10% skimmed milk in tubes. Each tube is inoculated with a lactic culture (1ml of bacterial inoculum in 10ml of skimmed milk). After incubation at 37°C, at an interval of 2 hours, 4 hours and 24 hours, the titration is carried out with Dornic soda (N/9) in the presence of 5 drops of phenolphthalein from each tube, until the temperature changes and pale pink color persists for at least 10 seconds. Acidity is determined by the formula: Acidity (°D) = VNaOH X 10 where VNaOH: Volume of NaOH used to extract the lactic acid contained in 10 ml of milk. The pH measurement is made directly by the pH meter, by dipping the electrode into the volume of the milk. The pH was determined each time the lactic acid dosage was carried out (Declomesnil, 2014).

Proteolytic power : The proteolytic activity of lactic acid bacteria was demonstrated and

compared on 1% milk agar. The bacteria to be tested, from a young culture, were seeded on the surface of these culture media by touch. After incubation at 37°C, the proteolytic activity of these bacteria was manifested by the appearance of a clear halo around the colonies (Benazzouz, 2012).

Lipolytic activity : This study contained two assays:

The activity lipolytic enzymes was assessed using a synthetic medium containing Tween 80. The presence of lipolytic enzymes was distinguished by the formation of a precipitate around the colonies due to the degradation of the salt of the fatty acid which constitutes Tween 80 (Tehreema *et al.,* 2011). After a regular interval of 24 hours of incubation, the Petri dishes were examined to evaluate this activity.

- Lecithinase activity was described by a technique which consists of enriching an ordinary agar by the incorporation of an egg yolk. The medium was presented in a Petri dish, inoculated by touches or making a central streak on the surface of the medium, and incubated at 37°C for 24 hours or more. Lecithinase activity was revealed by opacifications of the colony (Guiraud, 2003; Tehreema *et al.,* 2011).

Results and Discussion

Isolation and characterization of lactic acid bacteria

Lactic acid bacteria were only isolated from the set in which wheat was mixed with distilled water. In the other set containing acetic acid and wheat, LAB was not found. This absence could be attributed to the mortality of all indigenous LAB following the addition of vinegar. After the incubation period, round, whitish and clearly visible colonies appeared on MRS agar (Fig. 2) but in broth, the growth of LAB was reflected as homogeneous turbidity.

The bacterial cultures were purified and identified. In all, 54 LAB were isolated belonging to 8 genera and 20 species whose distribution according to the percentage of appearance is illustrated in Fig. 3.

The results also showed that there is a great diversity of bacteria between the grain samples used in this study. Canadian wheat showed maximum diversity of LAB with seven genera. LAB on the durum wheat of Algeria and French soft wheat showed the presence of only four genera. Three of these genera were common to both with one each being different (Fig. 4). LAB of the genera *Weisella* was found only on the French soft wheat flour which was absent in the samples of French soft wheat. These differences can be attributed to the culture and production conditions of each sample.

The variability in LAB from Moroccan flour and fermented Algerian durum wheat of the Hamoum type was also reported by Ennadir *et al.* (2014) and Mokhtari (2014). According to Sharma *et al.* (2019), 26 lactic acid isolates were obtained from 50 different samples of the traditionally fermented wheat flour dough known as "babroo" collected from different locations in the Himalayan province (Himachal Pradesh). Tahlaàti *et al.* (2018) identified 39 isolates of LAB from fermented wheat which belonged mainly to *Lactobacillus* (69%) followed by *Pediococcus* (15%), *Leuconostoc* (8%) and *Enterococcus* (8%).

Nineteen biochemical and physiological parameters of all the 54 species were studied (Fig. 5 and 6). None of the species showed

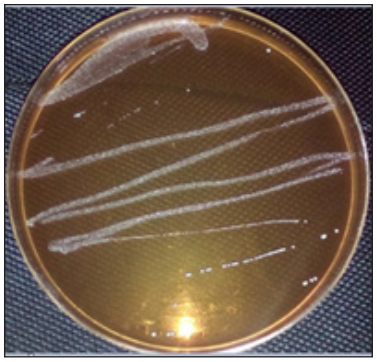

Fig. 2. Macroscopic appearance of lactic acid bacteria isolated.

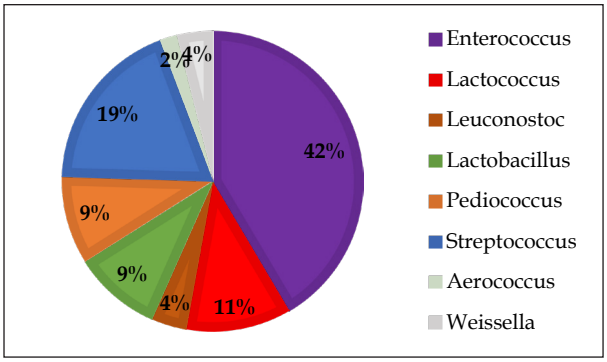

Fig. 3. Distribution of lactic acid bacteria in % in wheat and flour.

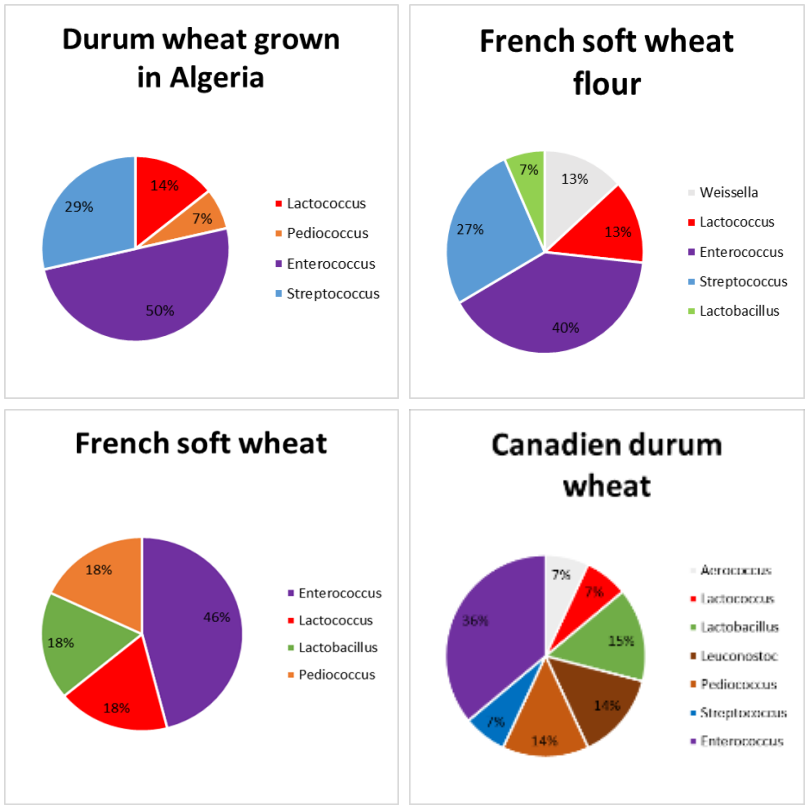

Fig. 4. Distribution of lactic acid bacteria in different wheat and flour samples.

positive test for oxidase, mannose, urease, nitrate reductase and hemolysis and hence not shown in Figures 5 and 6.

The results showed variations in immobility on mannitol, presence of catalase, O-nitrophenyl-β-D-galactopyranoside, Arginine dihydrolase, Lysine decarboxylase and Ornitine decarboxylase. Lactic acid bacteria are homofermentative except for certain strains which is heterofermentative. They used almost all the sugars in the process of the fermentation: galactose, sucrose, fructose, sorbitol, xylose, maltose. By the use of TSI medium, we had found that these strains preferred lactose and sucrose over glucose. In that, they are facultative anaerobe or anaerobic. All the lactic acid bacteria are sensitive to the tellurite and only 19 strains survived at 65°C. In the hostile environments viz. in presence of NaCl 2, 4 and 6, 5% and pH 4.0 the growth was slower than at pH 6.5. 53 strains produce the acetoin and one *Aerococcus* ssp. B'_{14} was exception. In this study, very few species showed growth in Sherman milk which contains 1% of methylene blue, an oxidizing environment. On sunflower milk, we had noted the growth of just five strains of lactic acid bacteria by coagulation of the casein.

All the 54 strains tested showed acidifying capacity, proteolytic nature and lipase activity. Only *Enterococcus acidominimus* b8 and b14; *Streptococcus thermophilus* b13, both strains of *Lactococcus lactis* ssp. *cremoris*; *Lactococcus lactis* ssp. *lactis*; *Leuconostoc mesenteroides* subsp. *mesenteroides* did not possess lacithinase activity. We studied acidifying acivity further as it is the most important characteristic. For all the bacteria tested, the production of lactic acid started slowly after 2 hours of incubation, which may correspond to the latent phase. The pH was determined each time. A lactic acid assay was carried out, including at time t = 0 h, all the lactic acid bacteria secrete a quantity of acid estimated at 10°D. (Acidity (°D) = VNaOH x 10). The best acidifying power was recorded after 24 hours in the strains: B'9: *Leuconostoc mesenteroides* subsp. *mesenteroides*, B'7: *Lactobacillus fermentum*, B7: *Lactococcus plantarum* with an amount of lactic acid estimated at 41°D, 40°D and 38°D, respectively, whose pH is varied between 5.63 and 6.69. B1: *Lactococcus diacetilactis*, F5: *Lactobacilus plantarum*, B"10: *Lactococcus lactis* subsp. *lactis*, B"7: *Lactobacillus rhamnosus*, F1: *Streptococcus thermophilus*, with an estimated

GN	Species	Strains	C	G	L	S	X	F	M	D	S	O	A
1	*Enterococcus acidominimus*	B2											
		B8											
		B9											
		B10											
		B11											
		B12											
		B14											
		B″3											
		B″4											
		B″5											
		B″8											
2	*Enterococcus saccharolyticus*	F2											
		F3											
		F4											
		F9											
		F10											
		F15											
		B′2											
		B′3											
		B′6											
		B′10											
		B′13											
		B″9											
7	*Streptococcus thermophilus*	F1											
		F6											
		F13											
		F14											
		B13											
		B′12											
		B3											
		B4											
		B5											
8	*Weissella confusa*	F11											
		F12											
	Aerococcus ssp.	B′14											
	Lactococcus lactis ssp. cremoris	F7											
		F8											
	L. plantarum	F5											
	L. brevis	B″1											
	L. casei	B′4											
	L. fermentum	B′7											
	L. rhamnosus	B″7											
	L. diacetilactis	B1											
	Lactococcus lactis ssp. *hordnaie*	B′5											
	L. lactis ssp. *lactis*	B″10											
	L. lactis ssp. *lactis*	B″11											
	L. plantarum	B7											
	L. mesenteroides subsp. dectranicum	B′8											
	L. mesenteroides subsp. mesenteroides	B′9											
	Pediococcus acidilactici	B′1											
		B″2											
		B″6											
	P. dextrinicus	B6											
	P. parvilus	B′11											

C: Catalase, G : Glucose, S: Sucrose, L : Lactose, X: Xylose, F : Fructose, M: Maltose, D: Dextrose, S: Sorbitol. O: O- Nitrophenyl-β-D-galactopyranoside, G: Gelatin, **

**Test of Oxidase, mannose, urease, nitrate reductase and hemolysis were negative for all species and were hence not shown

Green color in box: positive test; Red color in box : negative test. Pink color in box: intermediate test

Fig. 5. Important biochemical properties of lactic acid bacteria.

		1	2	3	4	5	6	7	8	9	10
Enterococcus acidominimus	B2										
	B8										
	B9										
	B10										
	B11										
	B12										
	B14										
	B″3										
	B″4										
	B″5										
	B″8										
E. saccharolyticus	F2										
	F3										
	F4										
	F9										
	F10										
	F15										
	B′2										
	B′3										
	B′6										
	B′10										
	B′13										
	B″9										
Streptococcus thermophilus	F1										
	F6										
	F13										
	F14										
	B13										
	B′12										
	B3										
	B4										
	B5										
Weissella confusa	F11										
	F12										
Aerococcus ssp.	B′14										
Lactococcus lactis ssp. cremoris	F7										
	F8										
L. brevis	B″1										
L. casei	B′4										
L. fermentum	B′7										
L. rhamnosus	B″7										
L. plantarum	F5										
L. diacetilactis	B1										
L. lactis ssp. hordnaie	B′5										
L. lactis ssp. lactis	B″10										
	B″11										
L. plantarum	B7										
L. mesenteroides subsp. dectranicum	B′8										
L. mesenteroides subsp. mesenteroides	B′9										
Pediococcus acidilactici	B′1										
	B″2										
	B″6										
P. dextrinicus	B6										
P. parvilus	B′11										

		11	12	13	14	15	16	17	18	19	20
Enterococcus acidominimus	B2										
	B8										
	B9										
	B10										
	B11										
	B12										
	B14										
	B″3										
	B″4										
	B″5										
	B″8										
E. saccharolyticus	F2										
	F3										
	F4										
	F9										
	F10										
	F15										
	B′2										
	B′3										
	B′6										
	B′10										
	B′13										
	B″9										
Streptococcus thermophilus	F1										
	F6										
	F13										
	F14										
	B13										
	B′12										
	B3										
	B4										
	B5										
Weissella confusa	F11										
	F12										
Aerococcus ssp.	B′14										
Lactococcus lactis ssp. cremoris	F7										
	F8										
L. brevis	B″1										
L. casei	B′4										
L. fermentum	B′7										
L. rhamnosus	B″7										
L. plantarum	F5										
L.diacetilactis	B1										
L. ssp. hordnaie	B′5										
L. lactis ssp. lactis	B″10										
	B″11										
L. plantarum	B7										
L. mesenteroides subsp. dectranicum	B′8										
L. mesenteroides subsp. mesenteroides	B′9										
Pediococcus acidilactici	B′1										
	B″2										
	B″6										
P. dextrinicus	B6										
P. parvilus	B′11										

1,2-Sherman Milk: Reduction -Orange Coagulation-Yellow; 3,4,5-Sunflower milk: Reduction -(Positive -orange, Negative yellow) Coagulation-(Positive -orange, Negative yellow), A-acidification (Positive -orange, Negative yellow); 6 - Arginine dihydrolase (Positive -orange, Negative yellow) 7- Lysine decarboxylase (Positive -orange, Negative yellow); 8 Ornitine decarboxylase (Positive -orange, Negative yellow); 9- Ornitine decarboxylase(Positive -orange, Negative yellow); 10-Triple sugar iron (Positive -orange, Negative yellow); 11- Homofermentary (Positive -orange, Negative yellow)-, 12- Heterofermentary(Positive -orange, Negative yellow), 13-Respiratory profile-facultive anaerobic (yellow), aerobic (Orange); Growth in hostile environment 14-NaCl 2%, 15--NaCl 4%, 16-NaCl 6%, 17-pH 4.0, 18-pH 6.5 (growth indicated by orange and no-growth by yellow color), 19-Thermoresistance (resistant -orange; susceptible -yellow); Tellurite resistance (resistant -orange; susceptible -yellow)

Fig. 6. Important physiological properties of lactic acid bacteria.

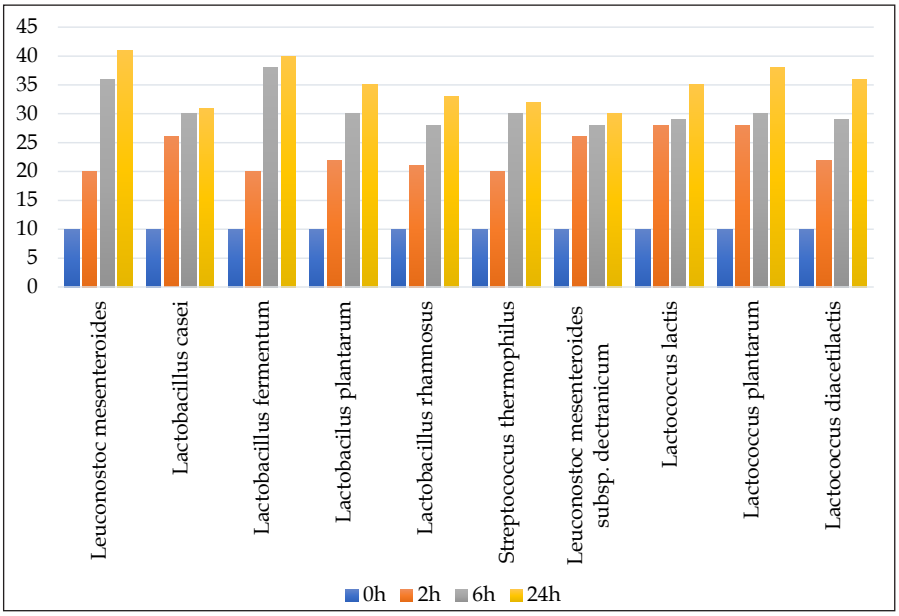

Fig. 7. Determination of acidifying activity as a function of time (0, 2, 6 and 24 hours) of the strains of lactic acid bacteria which have the best acidifying activity.

amount of lactic acid at 36°D, 35°D, 35°D, 33°D, 32°D respectively, B'4: *Lactobacillus casei* and B'8: *Leuconostoc mesenteroides* subsp. *dectranicum* with an estimated amount of lactic acid of 31°D and 30°D, respectively (Fig. 7). In our study, the strains of *Leuconoctoc mesenteroides* (subsp. *mesenteroides* and subsp. *dectranicum*) produced maximum lactic acid.

Conclusions

Through this study we have isolated and identified lactic acid bacteria from cereals (wheat) and flour. We were able to isolate 54 lactic acid bacteria belonging to eight genera. All isolated strains possessed acidifying, proteolytic and lipolytic activities. According to the results obtained during this study, we can conclude that cereals and flour have a biodiversity rich in lactic acid bacteria. The species which have maximum characteristics similar to *Leuconostoc mesenteroides* subsp. *mesenteroides* will be most suitable LAB for fermentation at industrial and household level.

Acknowledgement

A very special thank goes to the director and staff of SIM for their help in carrying out this work.

Conflict of interest

No conflict.

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Printed in March 2024