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Lactic Acid Bacteria Diversity in the Fermented Wheat Hamoum in West Algeria

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Abstract: The Algerian couscous called Hamoum is prepared from fermented wheat. Historically, the traditional fermented wheat Hamoum (BFH) was considered a food with medicinal properties in the prevention and treatment of many intestinal pathological and physiological complications. This product comes from the storage of wheat for longer than 12 months in an artisanal underground granary called a "Matmora". The fermented wheat product is rich with microorganisms beneficial to health. A microbiological study based on biochemical tests and physiological traits of the endogenous bacterial flora identified 42 lactic acid bacteria strains at different rates (27 lactobacilli with a rate of 65% and 15 lactococci with a rate of 35%). The bacterial strains were *Pediococcus pentosuceus* 1 (4.8%), *Pediococcus acidilactici* (7.5%), *Streptococcus bovis* (4.8%), *Streptococcus thermophilus* (4.8%), *Lactococcus* lactis ssp. lactis 1 (7.5%), *Lactococcus raffinolactis* (4.8%), *Lactobacillus pentossus* (4.8%), *Lactobacillus plantarum* (42%), *Lactobacillus brevis* (7.5%), *Lactobacillus* ssp. *paracasie paracasie* 3 (3.5%), *Lactobacillus* ssp. *paracasie* 1 (4.5%) and *Weissella confusa* (2.5%). Most of the lactic acid bacterial strains showed efficient amylolytic and proteolytic activity. Thus, naturally fermented wheat could be used as a dietary adjuvant as a preventive measure against intestinal pathological complications. Additional studies are underway to understand the cellular mechanisms arising from the use of BFH.

Key words: Fermented wheat hamoum, lactic acid bacteria

INTRODUCTION

Since the dawn of time, wheat has been a raw component of human food. It is rich in starch and has a protein content of approximately 10%. It is also a source of dietary fibre and micronutrients (i.e., vitamins and minerals); these nutritional ingredients represent a significant proportion of the recommended dietary allowances and the basis of the food pyramid. It is a staple food for humans and pets in many countries of the Maghreb.

Cereal-based fermented food products have a long history as products with beneficial properties. In food technology, lactic acid bacteria may contribute to the extension of the shelf life of products by producing various antimicrobial substances, including bacteriocins, lactic acid, hydrogen peroxide, fatty acids, diacetyl and other low molecular weight compounds during fermentation (Todorov *et al*., 2015).

Throughout history, the storage of wheat grain has provided mankind with a cushion against crop failure during periods of food shortage (Druvefors and Schnurer, 2004). Efforts to improve storage conditions include traditional techniques (in underground structures) to modern silos. However, the different storage techniques reveal that a surprising component of the benefits of wheat arise from contact with the ground triggering a natural fermentation process. Consumption of this naturally fermented wheat seems to confer preventive and/or curative effects against certain patho-physiological intestinal complications.

A traditional storage technique widely used in the Maghrebrural grain-growing regions in general and in Algeria in particular is called Matmora. This is an underground warehouse for the storage of wheat grains. During underground storage, the part of the wheat in direct contact with the ground undergoes metabolic transformation. Under these physico-chemical and microbiological conditions; the wheat acquires microbial flora with natural fermentation characteristics. This microbial flora, primarily lactic acid bacteria, has beneficial properties against certain digestive pathophysiological complications. A few studies have shown its nutritional and dietary benefits. The choice of the fermented wheat type Hamoum is based on several

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criteria. It was considered by our ancestors as a nutraceutical against certain digestive pathophysiological complications. Today, it is widely used as a health food in some rural areas.

During the storage period, the balance of endogenous microorganisms in the fermenting wheat grains can be affected by many factors, such as soil quality and weather conditions (mainly temperature and humidity), contributing to the emergence and growth of certain bacteria and yeasts responsible for the fermentation of grains (Yao *et al*., 2009). These microorganisms need energy to grow and wheat is an excellent substrate due to its richness in nutrients (carbohydrates, fats and proteins). However, access to these macronutrients requires microbial enzyme mechanisms (amylolytic, lipolytic and proteolytic) to convert them into bioavailable elements for the microorganisms.

Scientific curiosity led us to explore the diversity in the endogenous bacterial flora of the fermented wheat type Hamoum. The objective of our work is to isolate, purify and identify the strains of lactic acid bacteria in our fermented wheat.

Do the lactic acid bacteria starch-based fermented foods of the Maghreb countries with traditional and/or craft uses have the genetic background associated with nutritional and probiotic functional properties of interest in health?

Conventional bacterial cultivation methods have shown that the microbial flora of the fermented wheat type Hamoum contains lactic acid bacteria. We were interested in identifying new and better adapted strains ensuring equity and better results. In the literature, there are no data identifying the lactic acid bacteria of traditional fermented wheat. In order to provide solutions for research and development in public health, we set a goal to provide new information on the endogenous lactic acid bacteria of fermented wheat Hamoum. Many studies selected strains of nutritional or technological interest (Songre-Ouattara *et al*., 2008). However, the relationship of the lactic bacteria microflora with humans has been little studied in the context of fermented starchy foods, especially in terms of potential health effects.

MATERIALS AND METHODS

The aim of our study was to determine the microbiological characteristics of fermented wheat Hamoum (FWH) by the isolation and identification of the endogenous lactic acid bacteria strains. The choice of FWH as the plant material is based on its wealth of lactic acid bacteria in its rich endogenous flora. The natural fermentation of these lactic acid bacteria provides medicinal and nutritional benefits.

Our sample came from a rural Mediterranean region of Mostaganem city in west Algeria (Fig. 1). Just after the harvest, part of the crop is stored in a field in an underground storage unit called a "Matmora", a very archaic traditional technique used to this day in some isolated areas of the Maghreb (Fig. 2). The Matmora is a pit dug in clay soil in a truncated spherical shape, usually on high ground or near the farm. After the harvest season, the wheat seeds are poured into the pit (Matmora), lined with hay and then covered with hay and earth. Subsequently, abundant water is poured into the pit. The water evaporates as the days go by under the heat of the sun.

After two years of storage, part of the wheat in contact with the ground may undergo fermentation in its natural state. The choice of this rural area is justified by the preservation of such traditional storage techniques and its use for therapeutic purposes against certain digestive pathological and physiological complications. This fermented wheat has chemical and microbiological characteristics that are very different from those of non-fermented wheat.

The FWH was taken under aseptic and hygienic conditions in sterile bags to avoid any contamination that may affect the endogenous bacterial flora. The sample was taken from the peripheral part of the Matmora in contact with the ground. The sample was stored in the dark in a refrigerator in a food package at 04°C until needed.

Experimental protocol

Isolation and purification: First, 40 g of FWH was diluted in a mortar with 10 ml of physiological water. The sample was crushed and soaked to make a stock solution ready for decimal dilutions.

The elective and selective isolation of lactic acid bacteria culture on several media was performed according to the methods described by Carr *et al*. (2002). In this method, 1 ml of each dilution is inoculated in the bulk of the solid media MRS and M17 (Merck, Darmstadt, Germany) to obtain well-separated colonies. After incubation at 37°C for 48 h under anaerobic conditions, a microscopic examination is performed after Gram staining. The shape of the bacterial isolates and their association modes are observed and recorded. Gram positive and catalase negative colonies are planted alternately on MRS (De Man, Rogosa and Sharpe) and M17 agar until purification. The purity of the strains is revealed by uniform colonies having the same appearance (colour, size and shape) (Guiraud, 2003; Idoui *et al*., 2009).

Short-term conservation of the bacterial strain is carried out on a MRS solid medium slant for lactic acid bacteria. After growth at the optimum temperature, the cultures are maintained at + 4°C and re-plated every 4 weeks. For long-term storage, the bacterial strains are immersed in a solution containing 70% skim milk (enriched with 0.5 g/l of yeast extract and 0.5 g/l of glucose) and 30% of glycerol in cryotubes for storage at -20°C (Samelis *et al*., 1994).

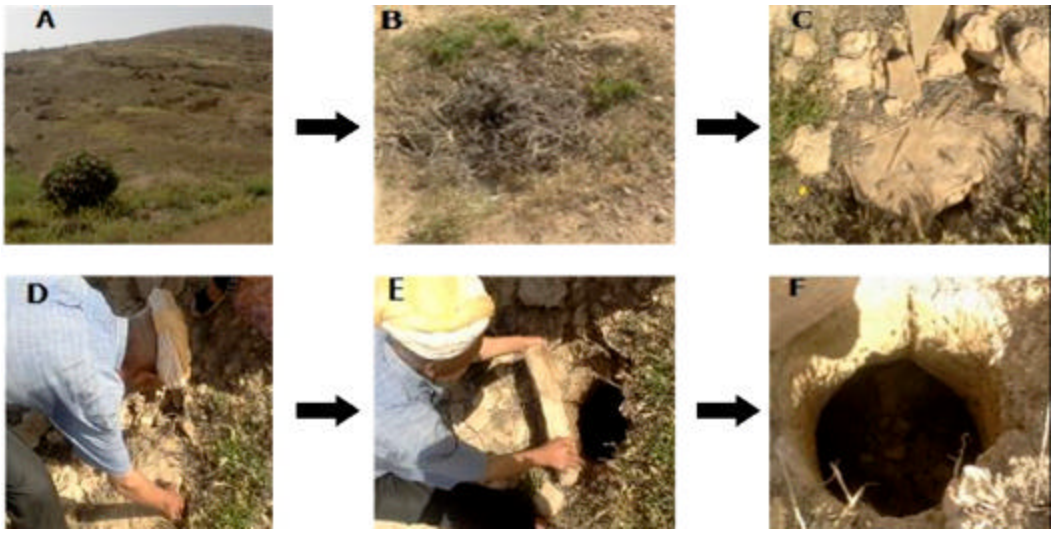

Fig. 1: Sample collection source of FWH from the Matmora in the West Algeria (Mostaganem city)

Fig. 2: Preparing an underground Matmora (Bartali and Debbarh, 1991)

Identification of physiological traits: Catalytic activity degrades hydrogen peroxide into oxygen and water. It is demonstrated by emulsifying one to two colonies of the isolate of the strain in a fresh solution of hydrogen peroxide. Abundant gas evolution as foam reflects the decomposition of hydrogen peroxide by the action of the tested enzyme (Marchal *et al*., 1991; Guiraud, 2003).

Rates of growth at different temperatures differentiate mesophilic and thermophilic lactic acid bacteria. The test is performed in M17 and MRS liquid media at different temperatures (15, 40 or 37°C) for 24 to 48 h. Cell growth is assessed by the presence of a disorder (Badis *et al*., 2005; Hariri *et al*., 2009).

Growth under hostile conditions is examined by growing the bacterial colonies in M17 and MRS media with 2, 4 or 6.5% NaCl. After incubation at 37°C for 24 h, the growth is assessed by the presence of a disorder.

The growth test in M17 and MRS liquid media is carried out at pH 5 and pH 9 for 24 to 48 h. Bacterial growth is assessed by the presence of a disorder at the bottom end of the tube (Hariri *et al*., 2009).

The growth test in different pH environments is performed in M17 and MRS liquid media at pH 5 and pH 9 for 24 to 48 h. Bacterial growth is assessed by the presence of a disorder of the medium at the bottom end of the tube.

The Knowledge test of fermentation type is performed in MRS medium in the test tubes. The medium containing colonies is poured into Durham bells to demonstrate the production of gas. After incubation at 37°C for 24 to 48 h, the presence or absence of gas in the bell indicates the fermentation type. The homo-fermentative strains will produce 90% lactic acid and only 10% CO_2, whereas the hetero-fermentative strains will produce lactic acid and CO_2 in equal proportions (Carr *et al*., 2002).

Heat resistance is evaluated in MRS liquid medium in a water bath at 63.5°C for 30 min. After quenching, the samples are incubated at 30± 1°C for 48 to 72 h. A positive result is a disorder (Badis *et al*., 2005).

Proteolytic activity is assessed in YMK medium (yeast milk agar) supplemented with sterile skimmed milk. The bacteria are cultured for 18 h in MRS medium and M17 and then seeded by touching the YMK medium and incubated at 37°C for 24 h. Colonies that give clear areas possess a proteolytic activity.

Lipolytic activity is demonstrated by bacterial culture in MRS medium and M17 with a seeding by touching the agar with Tween 80 (agar added to 2.5 ml of sterile Tween 80) and incubated at 37°C for 24 h. The identification of amylolytic activity is based on a pre-culture of our bacterial strains for 18 h; the isolates were seeded by touching MRS medium-A (substituting 10 g of

lactose with 20 g of soluble starch). After incubation at 30°C for 48 h, bacterial colonies were exposed to a solution of Lugol. Positive results are the presence of a clear area around bacterial colonies (Thapa *et al*., 2006).

Determination of biochemical tests

Mannitol-mobility test: The technique consists of growing bacterial strains in mannitol-Mobility medium after incubation at 37°C for 24 h. Fermentation of mannitol resulted in a change of the culture medium to yellow (Guiraud, 2003).

TSI Agar (Glucose Lactose Sucrose-H2S): Using a handle containing sampled colonies, we seeded the slope and the residue of a tube. After incubation at 30±1°C for 48 h to 72 h, there is a yellow colouring indicator at the slope of the test tube indicating positive for lactose, at the pellet end indicating positive for glucose and in the intermediate area indicating positive for sucrose. This test also allows the production of H2S (blackening of the area joining the slope and the pellet) and gas (bubbles in the agar).

Search for ADH: The bacterial strains were grown in Moeller arginine agar after incubation at 37°C for 24 h. A positive culture is identified by a change to yellow due to glucose metabolism. Degradation of arginine and the release of ammonia prevent the change to yellow.

Test of simmons citrate: This medium does not contain citrate as the sole carbon source; only bacteria with citrate permease are able to grow on this medium. The middle of the slant is seeded in a longitudinal groove by means of a handle containing a colony and the slant is incubated at 30±1°C for 5 days. A positive citrate result is manifested by alkalization of the medium (colour change of medium indicator to blue). A negative citrate result is manifested by the absence of bacterial growth (green colour, unchanged medium).

Production of acetoin: The bacterial strains are inoculated in Clark and Lubs media to test for the production of acetoin. After incubation at 37°C for 24 h, 1 ml of culture is deposited together with 0.5 ml of reagent a-naphthol in 6% of absolute alcohol (VP1) and 0.5 ml of a solution (NaOH 16% in distilled water (VP2)) in a haemolysis tube to carry out the Voges-Proskaeur reaction. The tube is thoroughly stirred and kept in touch with the free area for 10 min at room temperature. The production of acetoin results in a pink ring on the surface of the yellow medium.

RESULTS AND DISCUSSION

Macroscopic and microscopic appearance: Isolates that do not exhibit the phenotypic characteristics of lactic acid bacteria (Gram positive, catalase negative and oxidase negative) were rejected. From 134 isolates, 42 colony forming units were selected, 16 colony forming units in M17 agar at a temperature of 30°C under aerobic conditions and 26 forming unit in MRS agar at a temperature of 37°C under anaerobic conditions. The bacterial colonies were purified by size, shape, regular or irregular edges and colour (Table 1, 1a and 1b).

Physiological and biochemical criteria: Physiological and biochemical criteria are based on the growth of lactic acid bacteria at different temperatures (15, 25 and 45°C), NaCl concentration, pH (5, 6 and 9), the type of fermentation, the bacterial synthesis of sugars such as TSI (glucose, lactose and sucrose), mannitol mobility and enzyme activity (ADH, citrate of Simmons and acetoin) (Table 2, 2a and 2b).

Physiological criteria: All the bacterial strains are catalase negative. The growth temperature for the bacterial strains ranges from 15 to 45°C, with an optimum temperature of 37°C. With the exception of strains FWH6, FWH8, FWH9, FWH11, FWH12, FWH13 and FWH14 cultured in MRS medium, all bacterial strains show growth at a temperature of 15°C in both M17 and MRS media. With the exception of strains FWH1, FWH7, FWH8, FWH11, FWH17, FWH19, FWH22, FWH26, FWH30, FWH32, FWH35, FWH38 and FWH39, all the bacterial strains grow at the temperature of 45°C in both MRS media and M17.

pH: The results show that the pH decreases less rapidly during the first hour; then, the lactic acid bacteria produce a larger amount of lactic acid. Virtually all bacterial strains grew at pH 6. However, some strains required pH 4 or pH 9.

NaCl: Only bacterial strains FWH1, FWH2, FWH7, FWH15, FWH17, FWH18, FWH21, FWH22, FWH30, FWH34 and FWH41 grow in culture media at 02, 04 and 06.5% NaCl, or Other. Strains grow either at 02 or 04% NaCl. Most of our strains grow at pH 5 and pH 6.

Fermentative type: With the exception of strains FWH6, FWH22, FWH23, FWH38 and FWH41, all bacterial strains are homo-fermentative. Most of the strains are heat resistant. We observed bacterial growth in M17 and MRS broth media after a heat treatment at 63.5°C for 30 min.

Sugars: The lactic acid bacteria mobility test allows the differentiation thereof. This test is determined by a colour change of the medium from red to yellow in the strains FWH1, FWH3, FWH7, FWH14, FWH15, FWH16, FWH17, FWH18, FWH19, FWH21, FWH22, FWH24, FWH28, FWH29, FWH31, FWH34, FWH35, FWH36, FWH40 and FWH41. This result is due to the acidification generated during the fermentation of mannitol and therefore these isolates ferment mannitol.

Table 1: Macroscopic and microscopic appearance of lactic acid bacteria strains in M17 agar

	Macroscopic characteristics			Microscopic characteristics	
Strains	Form	Size	Colour	Form	Mode of association
FWH1	Spherical	Small	Whitish	Bacilli	In clusters isolated
FWH2	Spherical	Small	Clear	Coccobacilli	Long and Short chain
FWH3	Spherical	Small	Whitish	Coccobacilli	In clusters Long chain
FWH4	Spherical	Small	Whitish	Coccobacilli	In clusters Long chain
FWH5	Spherical	Small	Whitish	Bacilli	In clusters isolated
FWH6	Spherical	Small	Whitish	Coccobacilli	in clusters short chain
FWH7	Lenticular	Very small	Clear	Coccobacilli	In clusters short isolated
FWH8	Lenticular	Very small	Clear	Hull	Isolated short chain
FWH9	Spherical	Small	Clear	Hull	In clusters
FWH10	Spherical	Very small	Yellowish	Hull	In clusters
FWH11	Spherical	Very small	Whitish	Hull	In clusters
FWH12	Spherical	Small	Whitish	Hull	In clusters
FWH13	Spherical	Very small	Whitish	Hull	In clusters
FWH14	Spherical	Very small	Whitish	Hull	Long and Short chain tetrad
FWH15	Spherical	Small	Yellowish	Hull	Short chain diplococcus
FWH16	Spherical	Small	Clear	Coccobacilli	Short chain diplococcus

Table 1a: Macroscopic and microscopic appearance of lactic acid bacteria strains in MRS agar

	Macroscopic characteristics			Microscopic characteristics	
Strains	Form	Size	Colour	Form	Mode of association
FWH 17	Spherical	Small	Whitish	Coccobacilli	Short chain isolated
FWH 18	Spherical	Small	Whitish	Coccobacilli	Short chain isolated
FWH 19	Spherical	Small	Whitish	Bacilli	Isolated pair
FWH 20	Spherical	Small	Whitish	Coccobacilli	Long chain
FWH 21	Spherical	Small	Whitish	Bacilli	In clusters
FWH 22	Spherical	Small	Whitish	Coccobacilli	Isolated in clusters short chain
FWH 23	Spherical	Small	Whitish	Coccobacilli	Isolated in clusters short chain
FWH 24	Spherical	Very small	Clear	Coccobacilli	Isolated in clusters short chain
FWH 25	Spherical	Small	Whitish	Coccobacilli	Isolated in clusters short chain
FWH 26	Spherical	Small	Whitish	Bacilli	In clusters
FWH 27	Spherical	Small	Whitish	Bacilli	In clusters
FWH 28	Spherical	Small	Whitish	Bacilli	isolated
FWH 29	Spherical	Small	Whitish	Bacilli	Isolated diplobacilli

Table 1b: Macroscopic and microscopic appearance of lactic acid bacteria strains in MRS agar

	Macroscopic characteristics			Microscopic characteristics	
Strains	Form	Size	Strains	Form	Size
FWH30	Spherical	Small	Whitish	Coccobacilli	Isolated pair
FWH31	Spherical	Small	Whitish	Coccobacilli	Isolated pair
FWH32	Spherical	Small	Whitish	Coccobacilli	chainette in pairs
FWH33	Spherical	Very small	Clear	Coccobacilli	chainette in pairs
FWH34	Spherical	Small	Whitish	Coccobacilli	chainette in pairs
FWH35	Spherical	Small	Whitish	hull	In short chain pair, V and Y
FWH36	Spherical	Very small	Clear	Bacilli	Diplococcus short chain
FWH37	Spherical	Very small	Clear	Bacilli	Isolated diplococcus
FWH38	Spherical	Small	Whitish	Shell	Isolated diplococcus in chainette
FWH39	Spherical	Small	Whitish	Hull	Isolated diplococcus in chainette
FWH40	Spherical	Small	Whitish	Hull	Isolated diplococcus in chainette
FWH41	Spherical	Small	Whitish	Hull	Isolated diplococcus in chainette
FWH42	Spherical	Small	Whitish	Hull	Isolated diplococcus in chainette

The fermentation of glucose, lactose and/or sucrose in 04 lactococci strains (FWH3, FWH8, FWH41, FWH42) and 12 *Lactobacillus* strains (FWH1, FWH18, FWH21, FWH22, FWH23, FWH25, FWH27, FWH28, FWH29, FWH30, FWH31, FWH34) is accompanied by the production of CO_2 and H_2S in the strain (FWH14).

After testing for the degradation of arginine (ADH), only 03 lactococci strains (FWH3, FWH10, FWH14) and one lactobacilli strain (S19) were identified with arginine dihydrolase function.

Simmons citrate use was positive for the strains FWH6, FWH9, FWH13, FWH18, FWH31, FWH34, FWH40 and FWH41.

The production of acetoin is observed in the strains, with the exception of FWH4, FWH7, FWH9, FWH12, FWH13, FWH14, FWH17, FWH22, FWH24, FWH26, FWH27, FWH30, FWH31, FWH32, FWH34, FWH37 and FWH39.

Ranking of isolates: After the various physiological and biochemical tests for identification, we found that our 42 bacterial strains belong to different bacterial genera and

Table 2: Physiological and biochemical profile of the lactic acid bacteria strains in M17 agar

		------ Different T° --------			-------- % of NaCl ----------			---------- pH ----------						---------- TSI ----------					
Strains	Cat	15°	37°	45°	2%	4%	6.5%	5	6	9	Hom /Het	63°/30 nm	Man	G	L	S	ADH	Cit	Acet
FWH1	-	+	+	-	+	+	+	-	+	+	Hom	+	+	+	+	+	-	-	+/-
FWH2	-	+	+	+	+	+	+	+	+	+	Hom	-	-	-	-	-	-	-	+
FWH3	-	+	+	+	+	+	-	+	+	+	Hom	+	+	+	+	+	+	-	+
FWH4	-	+	+	+	+	+	-	+	+	+	Het	+	-	+	+	-	-	-	-
FWH5	-	+	+	+	+	+	-	+	+	+	Hom	+	-	-	-	-	-	+/-	+
FWH6	-	-	+	+	-	-	-	-	+	+	Het	+	-	-	-		-	+	+
FWH7	-	+	+	-	+	+	+	-	+	+	Het	+	+	+	+	-	-	-	-
FWH8	-	-	+	-	+	+	-	-	+	+	Hom	+	-	+	+	+	-	-	+
FWH9	-	-	+	+	-	-	-	-	+	-	Hom	+	-	-	-	-	-	+	-
FWH10	-	+	+	+	-	-	-	-	+	-	Hom	+	-	-	-	-	+	-	+
FWH11	-	-	+	-	-	-	-	-	+	-	Hom	+	-	-	-	-	-	-	+/-
FWH12	-	-	+	+	-	-	+	+	+	-	Hom	+	-	-	-	-	+/-	-	-
FWH13	-	-	+	+	-	-	-	-	+	-	Hom	+	-	-	+	+	-	+	-
FWH14	-	-	+	+	-	-	-	-	+	-	Hom	+	+	-	+	+	+	-	-
FWH15	-	+	+	+	+	+	+	-	+	+	Hom	+	+	+	+	-	-	-	+
FWH16	-	+	+	+	+	+	-	+	+	+	Hom	+	+	+	+	-	-	-	+

Cat: catalase, Hom: Homo-fermentative, Het: hetero-fermentative; 63°/30 mn: heat resistance, Man: Mannitol mobility, TSI: (Agar Glucose Lactose-Sucrose-H2S) (G: glucose, L: lactose, S: sucrose), ADH: Arginine dihydrolase, Cit: Simmons citrate, Acet: Acetoin

Table 2a: Physiological and biochemical profile of the lactic acid bacteria strains in MRS agar

		----- Different T° -------			-------- % of NaCl ----------			---------- pH ----------						---------- TSI ----------					
Strains	Cat	15°	37°	45°	2%	4%	6.5%	5	6	9	Hom /Het	63°/30 nm	Man	G	L	S	ADH	Cit	Acet
FWH 17	-	+	+	-	+	+	+	-	+	+	Hom	+	+	-	-	-	-	-	-
FWH 18	-	+	+	+	+	+	+	+	+	+	Hom	-	+	+	+	+	-	+	+
FWH 19	-	+	+	-	+	+	-	+	+	+	Hom	+	+	-	+	+	+	-	+
FWH 20	-	+	+	+	-	-	-	+	+	-	Hom	-	-	-	+	+	-	-	+
FWH 21	-	+	+	+	+	+	+	-	+	-	Hom	-	+	+	+	+	-	-	+
FWH 22	-	+	+	-	+	+	+	-	+	+	Het	-	+	+	+	+	-	-	-
FWH 23	-	+	+	+/-	+	-	+	-	+	-	Het	-	-	+	+	+	-	-	+
FWH 24	-	+	+	+	-	-	+	+	+	+	Hom	+	-	-	-	-	-	-	-
FWH 25	-	+/-	+	+	-	-	-	+	+	+	Hom	-	+	+	+	+	-	-	+
FWH 26	-	+	+	-	+	-	-	+	+	+	Hom	-	-	-	-	-	-	-	-
FWH 27	-	+/-	+	+	+	+	-	+	+	+	Hom	-	+	+	+	+	-	-	-
FWH 28	-	+	+	+	+	-	-	+	+	+	Hom	+	+	+	+	+	-	-	+
FWH 29	-	+	+	+	+	-	-	+	+	-	Hom	+	+	+	+	+	+/-	-	+

Cat: catalase, Hom: Homo-fermentative, Het: hetero-fermentative, 63°/30 mn: heat resistance, Man: Mannitol mobility, TSI: (Agar Glucose Lactose-Sucrose-H2S) (G: glucose, L: lactose, S: sucrose), ADH: Arginine dihydrolase, Cit: Simmons citrate, Acet: Acetoin

Table 2b: Physiological and biochemical profile of the lactic acid bacteria strains in MRS agar

		---- Different T° ----			------ % of NaCl ------		---------- pH ----------					---------- TSI ----------					
Strains	Cat	15°	37°	45°	2%	4%	6.5%	5	6	9	Hom /Het	63°/30 nm	Man	G	L	S	ADH	Cit	Acet
FWH30	-	+	+	-	+	+	+	+	+	+	Hom	-	-	+	+	+	-	-	-
FWH31	-	+	+	+	+	+	-	+	+	+	Hom	-	+	+	+	+	-	+	-
FWH32	-	+	+	-	+	+	-	-	+	-	Hom	-	-	-	-	-	-	-	-
FWH33	-	+	+	+	+	+	-	-	+	-	Hom	-	-	-	-	-	-	-	+
FWH34	-	+	+	+	+	+	+	+	+	+	Hom	+/-	+	+	+	+	-	+	-
FWH35	-	+	+	-	-	+	+	+	+	+	Hom	-	+	-	-	-	-	-	+
FWH36	-	+	+	+/-	+	+	+	-	+	+	Hom	+	+	-	-	+	-	-	+
FWH37	-	+	+	+	-	-	-	-	+	-	Het	+/-	-	-	-	-	-	-	-
FWH38	-	+/-	+	-	-	-	-	-	+	+	Hem	-	-	-	-	+	-	-	+
FWH39	-	+	+	-	+	-	-	+	+	-	Hom	+	-	-	-	+	-	-	-
FWH40	-	+/-	+	+	-	-	-	+	+	+	Hom	-	+	+	+	+	-	+	+
FWH41	-	+	+	+	+	+	+	+	+	+	Het	-	+	+	+	+	-	+	+
FWH42	-	+	+	+	-	-	-	+/-	+	+	Hom	-	-	+	+	+	-	-	+

Cat: catalase; Hom: Homo-fermentative, Het: hetero-fermentative; 63°/30 mn: heat resistance, Man: Mannitol mobility; TSI: (Agar Glucose Lactose-Sucrose-H2S) (G: glucose, L: lactose, S: sucrose); ADH: Arginine dihydrolase, Cit: Simmons citrate; Acet: Acetoin

species, with lactobacilli 27 with a rate of 65% and 15 lactococci with a rate of 35%. Robert *et al.* (1999) identified a variety of LAB microflora in traditional French wheat sourdough with important *Coccobacilli* and *Lactobacilli.*

Our results show that the FWH9 and FWH42 strains belong to the species *Pediococcus pentosuceus* 1 with a rate of 4.8%; the strains FWH10, FWH11, FWH17 and FWH41 belong to the species *Pediococcus acidilactici* with a rate of 7.5%; FWH14 and FWH18 strains belong to the species *Streptococcus bovis* with a rate of 04.8%; FWH12 and FWH13 strains belong to *Streptococcus thermophilus* with a rate of 4.8%; the strains FWH3, FWH16 and FWH31 belong to the species *Lactococcus lactis* ssp. lactis 1 with a rate of 7.5%; FWH4 and FWH8 strain belong to the species *Lactococcus raffinolactis* with a rate of 4.8%; the strains FWH1 and FWH7 belong to the species *Lactobacillus pentossus* with a rate of 4.8%; the strains FWH2, FWH5, FWH6, FWH18, FWH20, FWH21, FWH24, FWH25, FWH27, FWH28, FWH29, FWH30, FWH32, FWH36, FWH37, FWH38 and FWH40 belong to the species *Lactobacillus plantarum* 1 with a rate of 42%; the strains FWH26, FWH35 and FWH39 belong to the species *Lactobacillus brevis* with a rate of 7.5%; S19 strains belong to the species *Lactobacillus paracasie* ssp. *paracasie 3* with a rate of 3.5%; the strains FWH22, FWH23 and FWH33 belong to the species *Lactobacillus* ssp. *paracasie* 1 with a rate of 4.5% and the FWH34 strain belong to the species *Weissella confusa* with a rate of 2.5% (Fig. 3).

Suitable strains need to be selected and efforts are needed to produce strains of LAB in FWH products that will be available for clinical studies. This can gauge the impact of probiotics on consumer nutrition and health and increase the number of people who can benefit (Franz *et al.*, 2014).

Discussion of the data: Wheat, as does any biological material through the stages of life and in certain environmental conditions, undergoes biochemical and physiological changes that can have beneficial effects on its useful value. The purpose of this study was to explore the bacterial flora of the lactic fermented wheat type Hamoum (FWH) stored in a natural underground storage unit called a Matmora. The FWH is considered by our elders as a traditional local product with therapeutic gastroenterological properties.

We used classical microbiological techniques to isolate and identify different types of lactic acid bacteria in the FWH. L. plantarum 1 was the dominant LAB species in wheat and legume sourdough (Rizzello *et al.*, 2014). It has been shown that the addition of legumes and the sourdough fermentation increased the concentration of functional compounds (e.g., essential free amino acids, phenols and dietary fibre). Fermented wheat rich in different LAB species leads to good nutritional, sensory and functional properties.

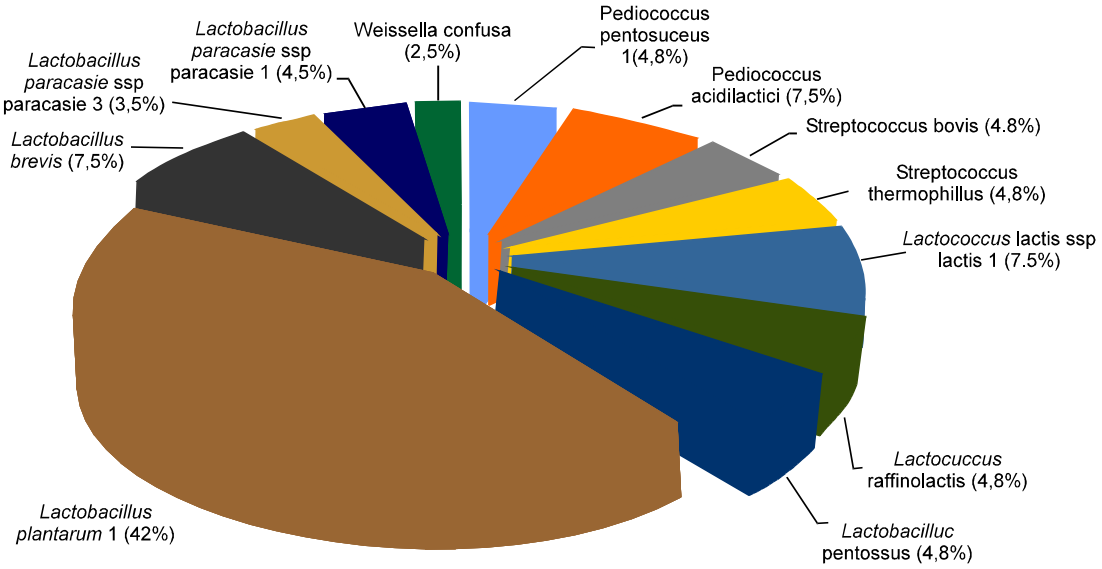

Fig. 3: Distribution of lactic acid bacteria strains in the FWH

Forty-two lactic acid bacteria strains were isolated and identified on MRS, M17 and MSE media from traditional FWH. Because of the nutritional requirements of these lactic acid bacteria, the culture media must be very selective and elective in terms of sugar content, nitrogenous materials and especially growth factors (Pilet *et al*., 2005). The isolates were all gram-positive and catalase-negative. Studies of major morphological, biochemical and physiological traits of the lactic microflora from the FWH were performed following the recommendations of similar studies (Carr *et al*., 2002; Guiraud, 2003; Axelsson, 2004; Katina *et al*., 2007). The isolates show genus and species diversity.

Unlike some studies of traditional fermented dairy products, such as Dahi in India where the lactococci rate (73%) is higher than the lactobacilli (27%) (Harun-ur-Rashid *et al*., 2007), our results showed a predominance of lactobacilli, with a rate of 65%, compared to lactococci, with a rate of 35%. This observation is explained by the fact that they grow in harsh conditions in the presence of 6.5% NaCl, pH 9.6, with a growth temperature at 45°C and usually survive 30 min at 60°C (Larpent *et al*., 1997). This confirms our results because our FWH comes from an environment requiring special storage conditions in the traditional unit (Matmora). In particular, the process of fermentation during storage renders the endogenous bacterial flora slightly acidic due to the production of lactic acid and possibly other acid metabolites such as volatile fatty acids. The study of the main morphological, biochemical and physiological characteristics showed a diversity and prevalence of lactic acid bacteria isolated from FWH. However, the results depend mainly on the nature of the biological material, geographic region, sampling techniques, isolation and identification and selective and elective culture media of lactic acid bacteria.

All of the isolated lactic acid bacteria comprise *Lactobacillus* species, predominantly plantarum, with a rate of 40%. The other bacterial species range from 4.7 to 7.1%. All of our bacterial strains are Gram-negative, catalase positive. The growth temperature of all bacterial strains ranges from 15 to 45°C, with an optimal temperature of 37°C. The majority of the lactic strains are grown in a culture medium at 02 or 04% NaCl. With the exception of some strains, all bacteria are homo fermentative lactic acid bacteria. Fermentation improves the nutritional value of wheat, primarily by an increase in the content of essential amino acids such as lysine, methionine and tryptophan (Adams, 1990). Almost all bacterial strains have a growth pH of 6. However, some strains require pH 9 or 4. This acidifying activity depends on the growth and metabolism of lactic acid bacteria; the amount of acid produced varies among the strains studied. The fermentative activity of the bacterial flora causes a decrease in pH with a simultaneous increase in acidity and accumulation of lactic organic acids. The metabolic activities of the various strains of lactic acid bacteria involved in the fermentation of wheat result in the production of short chain fatty acids such as lactic acid, acetic acid, butyric acid and propionic acid (Kohajdova and Karovicova, 2007). Most of the strains are heat resistant, due to their confinement in the underground storage unit (Matmora), where the temperature is approximately 65°C. Only 03 strains (S3, S10, S14 and S19) synthesize the degrading enzyme arginine (ADH).

Our lactic acid bacteria do not produce glycan and are unable to degrade gelatin. The biochemical profile of the various types of bacterial sugars for possible identification of bacterial strains at species or subspecies level is complicated and tedious. The degradation of primary sugars such as glucose, lactose and sucrose depends on the surrounding environment; namely, the nature of the soil and the wheat. All of our bacterial strains possess proteolytic, amylolytic and lipolytic activity. Fermentation of our FWH enables an improvement in digestibility and starch degradation related to the enzymatic properties of lactic acid bacteria. The overall results of our study are a first approach to an initial characterization of the endogenous bacterial flora of FWH by conventional microbiological techniques in order to reveal the good nutritional virtues of the unknown traditional fermented wheat in some regions of Algeria. Lactic acid fermentation is a process which not only improves the organoleptic and hygienic quality but also the nutritional quality of food, especially preservation (Ampe *et al*., 1999). Our LAB strains could have a fermentative potential even at an industrial scale, as shown by Pruckler *et al*. (2015) with new LAB strains such as *L. brevis*, *L. sanfranciscensis*, *L. plantarum* and *L. pentossus*. Because of the anti-staling effect and the synthesis of antifungal compounds, wheat germ fermented by sourdough lactic acid bacteria may be considered as an ingredient to naturally extend bread shelf-life (Rizzello *et al*., 2011). FWH could be used in extending the preservation of bread and its derivatives at an industrial level.

Other microorganisms, such as Enterobacteriaceae, yeasts and moulds, were isolated from many cereal-based fermented foods and can influence their quality (Ashenafi, 2006). Traditional fermented wheat (FWH) with its endogenous bacterial ingredients could be a functional food and may constitute a biomedicine. According to the NUOP (National Union of pharmacy operators), bio-drugs are used today worldwide for the treatment of a number of pathologies. It also constitutes a major development for the pharmaceutical industry; the global biomedical market is estimated in 2015 at 200 billion US dollars. Biomedicines are carriers of innovative therapeutic solutions for severe diseases with no satisfactory treatment.

It has been shown that LFWGE (Fermented wheat germ extract *Lactobacillus* sp) could induce subcutaneous transplantation tumour apoptosis in nude mice and could act as a natural nutrient supplement or chemo-preventive agent in the treatment of human colon cancer (Zhang *et al*., 2015).

A fermented wheat germ extract (Avemar) has been demonstrated to inhibit metastatic tumour spread and prolong survival in colorectal cancer and melanoma patients and Breast Cancer Cell Proliferation and Invasion in Vitro (Anonym).

This study will initiate molecular studies for further characterization and evaluation.

Conclusion: Formerly, fermented wheat was considered a food with highly appreciated medicinal properties. BFH represents a new source of active compounds by its wealth of lactic acid bacteria and could open a wide range of research and application of new strains of lactic acid bacteria at the scale of industrial biotechnology. Our study has allowed us to isolate, identify and purify 42 lactic acid bacterial strains at different proportions: 62% of *Lactobacillus* sp, 14% of *Pediococcus* sp, *Streptococcus* sp 10 and 10% and 2 *Lactococcus* sp 04% of *Enterococcus* sp. Thus, naturally fermented wheat could be used as dietary adjuvant and diet as a preventive measure against intestinal pathological complications. Studies are underway to understand the cellular mechanisms important for its application. This study also serves to initiate molecular studies for further characterization and evaluation.

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