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Isolation and identification of dominant osmophilic *Leuconostoc* strains from traditional date product "Btana"

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Abstract

The current study aimed to isolate and identify dominant osmophilic bacteria associated with a traditional date product named "Btana", produced in south region of Maghreb countries. Samples were randomly collected after two month of storage from tow villages (*Mtarfat* and *Abani*) in the Algerian southern department "Adrar". A high osmotic pressure medium (MSE) was used for isolation of osmophilic bacteria, which were purified and examined for macroscopic and microscopic shape, Gram stain, catatalse, oxydase, acetoine and ADH production, reduction of nitrate, and motility. Isolates were then subculture on MRS medium for production of dextran, gas from glucose, growth in the presence of NaCl (3, 6.5 %) and sucrose (10, 20, 30, 40, and 50 %), pH tolerance (4.8, 6.5), growth temperature (10, 37, and 45°C) and thermo resistance (55°C for 15 min), enzymatic activity (proteolytic, lipolytic, hemolysis). Isolates were identified to specie's level by sugar fermentation. Their growth and acidification kinetic were also studied. Results identified two species of *Leuconostoc*; *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Leuconostoc mesenteroides* subsp. *dextranicum*. They show a high antibacterial activity against four indicator bacteria; *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

Introduction

Btana is a traditional date product in the south region of Algeria. It is a very popular food for the inhabitants of *Beni Abbas*, *Touat*, *Gourrarra*, *Tidikilt*, *Ziban*, *Oued righ*, *Ouad souf* and *Mezzab regions.* The product is resulted from date's traditional transformation that varies according to the region of production. However the basic transformation includes cleaning up; amalgamating and mixing dates to obtain a paste which is usually piled up in proper plastic bags or in linen bags or very originally into an old goat skin. The resulted paste is piled by layers, and a small pressure is established within the layers to homogenate dates repartition and to exclude the residual air from the bag. A small amount of a grind mixture of dried aromatic plants like basilica (*Ocinum basilicum*), and Juniperus (*Juniperus phonicea*), is dispersed on date paste in order to give an agreeable smell and taste to dates. After filling, bags are attached and closed hermetically. An exposure to sun helps to perform the quality of the paste which takes a good consistence. Then Btana is stored in a dry place away from insects and stock destructors. After 15 to one month of preservation dates would be ready for consumption, however the preservation period may take up to one year depending on inhabitants needs.

Btana, play a major role in the diet of population living in the regions mentioned above, it is widely and daily consumed especially in the off season of ripen dates. Furthermore Btana consumption rises practically during the holy month of Ramadan, popular festivities, funerals and to welcome guests. Today Btana is a small business activity offering some revenues to household artisan throughout the centre, the easterner and westerner south of Algeria. Nutritional value of Btana is undisputed since it originates from dates, which are rich in sugar (44–88%) and fiber (6.4–11.5%) minerals, vitamins and contain (0.2–0.4 %) of fats and a protein content (2.3–5.6%) higher than other major cultivated fruits such as apples, oranges, bananas and grapes which contain 0.3%, 0.7%, 1.0% and 1.0% of protein, respectively (Elleuch *et al*., 2008).

Other authors have found that dates are a high source of antioxidants, anthocyanins, carotenoids and phenols (Allane and Benamara, 2010). Besides chemical composition of dates that were significantly studied, microbial biodiversity of this fruit remain very scarcely (Hasnaoui *et al.*, 2010).

Although no studies hitherto have investigated the traditional date product "Btana". In literature, a

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number of traditional foods have gained importance, and many studies were carried out to assess their microbial diversity as well as the impacts of dominant micro flora on food qualities (Bonomo *et al*., 2010). Other studies showed that microbial strains isolated from local products display a variety of characteristics, and play an important role in their natural environments (Ouadghiri *et al.*, 2005). Thus, research is needed to investigate and to highlight the microbial biodiversity of Btana during storage for understanding the impact of the indigene micro flora on nutritional, organoleptic and safety of dates.

Our preliminary investigation concluded that Btana processing comes down in low hygienic conditions using relatively simple techniques and equipments which introduce an indigene microflora in Btana. The outcome of this micro flora depends mainly on its resistance to conditions established in Btana medium. These factors include anaerobiosis, low water activity and a strength osmotic pressure. Only micro flora which overcomes these conditions might survive to play a a key role in the final product. We have carried out a preliminary study that demonstrated a concomitant decrease of pH from 5.63 to 5.06 (average value) in Btana sampled within an internship periods before consumption (Abekhti *et al*., 2013). This finding might proved that Btana preservation undergone a fermentation process either by lactic or alcoholic nature. Subsequently a number of theories have been developed to elucidate the real biologic process occurred during Btana preservation.

Regarding the nature of Btana product, our intention was paid to bacterial group that can survive in rich sugar environment, especially to *Leuconostoc* species, which are a lactic acid bacteria exhibiting a high tolerance to osmotic pressure up to 50-60% (sucrose) which allows the bacteria to grow in syrup, liquid cake and other rich sugar environment (Justé *et al*., 2008d). *Leuconostoc* is the most predominant lactic acid bacteria inhabitant plants products, sugar refining plants, fruit mashes and some traditional African beverages (Vihavainen *et al.*, 2008; Ehrmann *et al.*, 2009).

Presence of *Leuconostocs* in the date product "Btana" is assayed while the food's conditions are thought to enhance its growth. Our hypothesis was verified by a search of *Leuconostoc* species in Btana samples using a selective medium and appropriate cultivation conditions. Moreover, the validation of our hypothesis required a more refined methodology that request for the safety and role of the isolated strains in the establishment of food proprieties as well as their role in its preservation. Therefore a battery of biochemical and physiological tests were carried out on the isolated *Leuconostoc* strains relying mainly on phenotypic characterizations. Adaptation of the bacterium to the harsh Btana environment was assayed by study of osmo-tolerance to various concentrations of sucrose broth. Growth characteristics were determined by study of the coupled growth-acidification curve. So far antimicrobial activities was screened by measure of the inhibitory effect against some indicator bacteria relevant to health consumer safety. Other technological propriety like enzymatic activity, thermo resistance, aroma production and dextran production were also studied.

Materials and Methods

Btana used in this study were produced by traditional transformation of dates belonging to a variety of *Hmira*. The fruits were harvested during the previous crop from *Mtarfat* and *Abani* towns in *Adrar* (south of Algeria), and have been stored till transformation to Btana. Samples were obtained after two month of storage and were used for strain isolation. For this purpose; 25 g of each sample are introduced into sterile stomacher bag containing 225 ml of tryptone salt broth (0, 1% tryptone, 0, 85% NaCl). Then, samples were blended for 1 min at ambient temperature in Stomacher device. Then 1 ml of the homogenized solution is suspended in 9 ml of TS broth, for serial dilutions. Isolation and dextran production were carried out by spread of 0.1 ml of each dilution on MSE agar plates with the following composition (g/l); Tryptone (10 g), gelatin (2.5 g), yeast extract (5 g), sucrose (100 g), glucose (5 g) sodium azide (70 mg), sodium citrate (1 g), agar (15 g), distilled water (1 l), pH 6, 8 (Mayeux *et al.*, 1962). Then plates were incubated for 72 h at 30°C.

Two uncrowded viscous colonies presumed to be *Leuconostoc* were picked up randomly from plates of each sample and purified by streaking on MRS plate containing: yeast extract (4 g/l), peptone (10 g/l), beef extract (8 g/l), triammonium citrate, (2 g/l), glucose 20 g/l, sodium acetate, 5 g/l; $MgSO_4 \cdot 7H_2O$ (0.1 g/l), $MnSO_4 \cdot H_2O$ (0.05 g/l), K_2HPO_4 (2 g/l), Tween 80 (1 cm^3/l); agar (15 g/l), and 1 l of distilled water, pH 6.8 (Mayeux *et al*., 1962).

Phenotypic characterization applied to identify the isolates was performed according to Hadadji *et al.* (2005) and Nieto-Arribas *et al.* (2010). Tests include; cell morphology, Gram staining, catalase, oxidase, nitrate reduction, respiratory type (fermentative or oxydatif), production of gas from glucose in MRS broth with inverted Durham tubes, production of ammonia from arginine by the method of Briggs, indole production, and Voges-Proskauer test in

Clark-Lubs broth. Physiological tests performed are: growth at 10, 37, and 45°C during three days in MRS broth, growth at 4.8 and 6.5 pH values during three days in MRS broth. Growth during three days in 3 and 6.5% NaCl MRS broth and growth in MRS broth supplemented with 30 µg/l of Vancomycin. All tests were performed after overnight cultivation of pure isolates checked by microscopic observation.

Carbohydrate fermentation

Ability of isolates to use various carbohydrates was investigated in modified MRS broth containing (0.04 g/l) of bromocresol purple dye (BCP) as a pH indicator and supplemented with 1% of one of the following carbohydrates: glucose, fructose, xylose, arabinose, galactose, mannitol, mannose, sucrose, trehalose, cellobiose, salicin, and melebiose as a sole carbon source. Anaerobic conditions are created by addition of sterile liquid paraffin (Hadadji *et al*., 2005).

Biotechnological activities

Isolates's thermo resistance was determined by inoculating and heating MRS broth at 60°C for 30 min. The viability of strains was verified by growth after 48 h of incubation at 30°C. Osmotolerance of the selected strains was assayed by growth on modified MRS broth (without glucose) supplemented by 10, 20, 30, 40, and 50% of sucrose. Milk coagulation propriety was determined by inoculating each strain in 9 ml of 10% skimmed milk supplemented with 0.5% of yeast extract. Tubes were incubated at 30°C for approximately 48 h or until coagulation occurred (Hadadji *et al*., 2005).

Enzymatic activity

The enzymatic activity of the isolates was assayed on media containing the target substrate. For proteolytic activity, strains were inoculated as spot on MRS agar supplemented with 10% of sterile skimmed milk. Casein hydrolysis is revealed by apparition of clear zone around the colonies, lipolytic activity is determined by strain streaking on nutrient agar containing tween 80 as sole lipids source and visualized by presence of a halo around the bacterial streak (Buffa *et al.*, 2005).

Hemolytic activity

Safety evaluation of strains was investigated by hemolytic activity on Muller-Hinton medium added with sheep blood. After 24 of incubation, the blood agar was controlled for hemolysis (Mami *et al*., 2008).

Antibacterial activity

The antibacterial activity was investigated by the agar-spot method described by Galvez *et al.* (2009), against *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* and *Bacillus subtilis* as target strains. Firstly, to check the antibacterial activity, the method of double layer reported by Mami *et al.* (2008) was used. For this purpose, the selected strains were spotted on surface of Muller-Hunton agar and incubated for 24 h at 30°C. Then each target strain was inoculated in a tube containing 7 ml of soft Muller Hunton agar (7.5 g/l agar instead of 15 g/l). After well homogenization, the soft agar was spread on surface of Muller-Hunton plate already cultivated with the assayed strains. Antibacterial activity was revealed by clear zone of growth inhibition around the spot. Strains were compared for their antibacterial activity by measurement of inhibition zone.

Study of growth and acidification curve

For growth study, individual colony of each strain was precultured overnight in 2 ml MRS broth at 30°C. Then 0.1 ml of the precultured was inoculated and vortexed in tube series of 10 ml MRS broth. Tubes were drawn from incubator each hour during 36 h and 0.1 ml of culture was used for absorbance measurements (Claire *et al.*, 1998). Isolates growth was simulating as batch culture performed in screw tubes at 30°C without agitation and monitored spectrophotometrically by absorbance measurements at 600 nm (A600). Values of OD (average of three measures) were converted to logarithm values and plotted into growth curves versus time (h). Serial dilutions were performed when needed. The specific growth rates (µ) of each strain were calculated from the generation times (g) obtained from slope of the straight-line part of the curve which correspond to the exponential phase, as recommended by Levata-Jovanovic and Sandine (1996). Simultaneously, pH of MRS broth was measured using a pH meter, and values were projected to draw the curve of acidification. Analyze of linear regression of pH, allowed us to determine mean acidification rate (Vmar) calculated by dpH/dt from the slope generated during the exponential growth phase between pH 6.2 and 5.2 (Bellengier *et al.*, 1997). Acidification rate is expressed by pH unity versus time (U $pH.min^{-1}$). pH minimal (pHm) achieved in MRS broth and the corresponding time (Tm, min) were also determined.

Results

Tow randomly isolates with dextransucrase activity were selected from each sample of Btana. The examined strains (AB_{04}, AB_{11}, MT_{03} and MT_{05}) were revealed Gram-positive, catalase and oxydase negative, chain and short chain-forming cocci and form white colonies on MRS agar and viscous colony on MSE agar plate (Figure 1). As shown in Table 1, the strains are facultative aeroanerobic and produce gas from glucose. Strains are unable to hydrolyze arginine, to reduce nitrate or to produce indole and acetone from glucose. They are resistant to Vancomycin (30 µg/l), mesophilic grow well at 10°C but not at 45°C. The fourth strains supported 3.5 % NaCl, but not 6% NaCl. No thermo resistance was observed. Strains support pH 6.5. Both enzymatic and hemolytic activities were not detected. However slight milk coagulation was observed after 48 h of incubation.

Results of sugar fermentation (Table 1) showed that the strains exhibit a little difference in their carbohydrate patterns. The strains MT_{03}, MT_{05} ferment actively, galactose, ribose, mannhose, xylose, lactose and glucose. But they are less active on aribinose and raffinose. The strain MT_{05} is distinguished by its weakly degradation of maltose. However the two strains are unable to produce acid from sorbitol and rhamnose. The other strains AB_{11} and AB_{04} are able to ferment sorbitol, maltose and raffinose. They degrade weakly galactose, ribose, mannose, rhamose, xylose and lactose and are totally inactive on arabinose. The four strains were unable to ferment mannitol and mellibiose.

Antibacterial activity was recorded for AB_{04}, AB_{11} and MT_{05}, almost MT_{03} had no antagonistic activity (Figure 2). A high inhibition effect was observed on *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC29212 and *Escherichia coli* ATCC 25922 by AB04 strain. A low antagonistic activity was screened on *Staphylococcus aureus* ATCC 25923. In comparison with AB_{04} strain, AB_{11} and MT_{05} exhibit a low antibacterial effect against *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC29212 (Figure 2, Table 2).

For all tested strains, a low antagonistic activity was screened on *Staphylococcus aureus* ATCC 25923. Although no antagonistic effect was observed on *Bacillus cereus* and *Bacillus subtilis*. Figure 3 shows that the initial growth of the tested bacteria is started without a remarkable delay. On the other hand a slight increase was shown from 0 h to up to 2 h, while an increased growth rate was started; except for MT_{03} which took 2 hour longer for growth acceleration (5 h). The maximum growth rate values observed during the exponential phase were 0.24, 0.26, 0.17, and 0.25 h^{-1} for AB_{04}, AB_{11}, MT_{03} and MT_{05}, respectively. Then

Table 1. Results of physiological and biochemical tests of AB_{04}, AB_{11}, MT_{03} and MT_{05} isolates

Tests		AB_{04}	AB_{11}	MT_{03}	MT_{05}
Catalase test		-	-	-	-
Acetoine Production (VP)		-	-	-	-
ADH		-	-	-	-
Thermo resistance at 63,5°C/30min		-	-	-	-
Growth at	10°C	+	+	+	+
	37°C	+	+	d	d
	45°C	-	-	-	-
Growth in pH	4,2	-	-	-	-
	4,8	-	-	-	-
	6,5	+	+	+	+
Growth with	3,5 % NaCl	+	+	+	+
	6,5 % NaCl	-	-	-	-
Milk coagulation		d	d	d	d
CO2 production in MRS broth		+	+	+	+
Nitrate reduction		-	-	-	-
Growth with Vancomycine 30µg/l		'	'	'	'
Dextran Production		+	+	+	+
Galactose		d	d	+	+
Ribose		d	d	+	+
Mannitol		-	-	-	-
Mannose		d	d	+	+
Sorbitol		+	+	-	-
Arabinose				d	d
Rhamose		d	d	-	-
Maltose		+	+	+	d
Xylose		d	d	+	+
Raffinose		+	+	d	d
Mellibiose		-	-	-	-
Lactose		d	d	+	+
Glucose		+	+	+	+

+: Positive reaction
-: Negative reaction
d: Delayed reaction

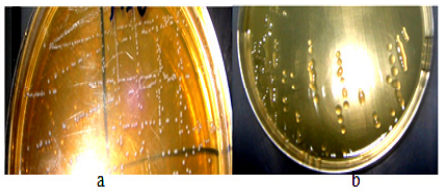

Figure 1. Macroscopic aspect of *Leuconostoc* strains on MRS (a) and MSE (b) agar plates.

Table 2. Inhibition zone of the targets bacteria

Indicator strains	Inhibition zone (mm)		
	AB_{04}	AB_{11}	MT_{05}
Enterococcus faecalis ATCC 29212	36	34	34
Escherichia coli ATCC 25922	34	33	33
Pseudomonas aeruginosa ATCC 27853	37	35	34
Staphylococcus aureus ATCC 25923	25	24	22
Bacillus subtilis	00	00	00
Bacillus cereus	00	00	00

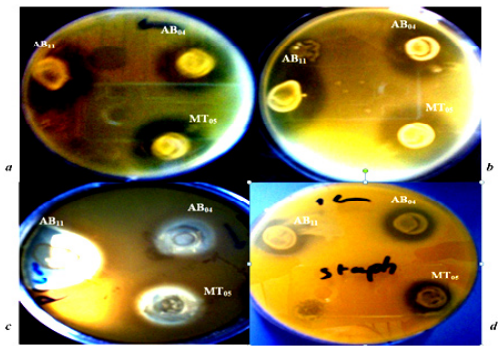

Figure 2. Antibacterial activity of AB04, AB11, MT05 against: a (*Enterococcus faecalis* ATCC 29212) b (*Escherichia coli* ATCC 25922), c (*Pseudomonas aeruginosa* ATCC 27853), d (*Staphylococcus aureus* ATCC 25923) on Muller-Hinton agar plate

Table 3. Kinetic and acidification parameters

Growth's factors	AB_{04}	AB_{11}	MT_{03}	MT_{05}
Vm(U pH.min-1)	0.40	0.49	0.37	0.57
Tvm(h)	5 - 7	5 - 7	5 - 7	5 - 7
pHm	4.24	4.26	4.24	4.45
TpHm(h)	28	26	28	26
µ (h-)	0.24	0.26	0.17	0.25
Generation time (min)	250	231	352	240

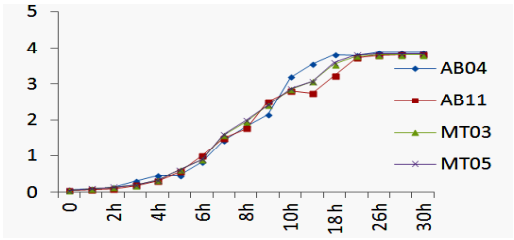

Figure 3. Growth kinetic of the isolates in MRS broth

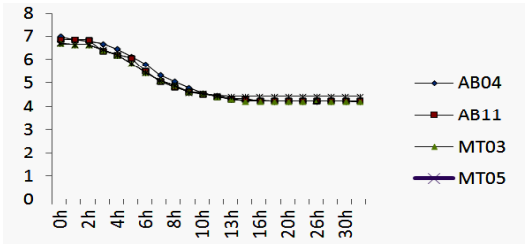

Figure 4. Acidification kinetic in MRS broth

strains enter the stationary phase after 8 h.

Inspections of the curves of acidification (Figure 4) shows that pH decrease according to growth time, moreover curves of the four strains are merely very superimpose. pH decrease is generally very slow in the first three hour, then it become faster. A fast and regular pH decrease is observed within the period (3 h-18 h), (2 h.30-18 h), (3 h.3-26 h), (3 h.30-13 h) for AB_{04}, AB_{11}, MT_{03}, MT_{05}, respectively. Between the fifth and seventh hour a linear slope accompanied pH decrease, we suggest that this period represent the exponential growth phase whilst mean acidification rate should be measured (Bellengier *et al*., 1997). Values of Vmar, pHm and Tm are presented in Table 3.

Discussion

Isolation and genus identification

The fourth strains (AB_{04}, AB_{11}, MT_{03}, MT_{05}) shared common phenotypic characteristics of lactic acid bacteria. Moreover, dextran activity, heteroferrmentative profile, resistance to 30 µg/ml Vancomycin and lack of L-arginine dihydrolase suggests strongly that the strains belong to the genus *Leuconostoc*. However, one could be confused with *Lactobacillus* genus that shared these characteristics with *Leuconostoc*, but species within the later genera are more coccid than *Lactobacillus* species (Antunes *et al.*, 2002). Previous studies have reported isolation of *Leuconostoc* species from rich sugar food like honey-dew of rye ear, sugar beet solution cane juice (Farrow *et al.*, 1989) and palm wine (Ehrmann *et al*., 2009). The remaining question is whether these strains survive or growing well in Btana as in other fermented vegetable products. It is well established that *Leuconostoc* is very fastidious bacterium that has requirements for multiple growth factors, preformed amino acids, purine and pyrimidine bases and many other nutrients (Björkroth and Holzapfel, 2006). Foucaud *et al.* (1996) had reported the essential amino acids and vitamins required for *Leuconostocs* growth which include cysteine, threonine, pantothenic, nicotinic acids whereas thiamine, riboflavin, thymidine, folic acid, and inosine were growth stimulatory. In fact some authors listed more than 17 amino acid present in dates among theme eight are essential present in date pulp and others are in date seeds (Vayalil, 2012).

Isolation of *Leuconostocs* from such product proves that Btana is a rich nutrient that can fulfill the nutritional requirements for growth of this microorganism. Perhaps, sugar concentration and anaerobiosis generated in Btana is also in favor of the isolated strains in detriment of others micro flora that make nutrient more available for these bacteria. Nevertheless, the output of these growth factors after date transformation to Btana is unknown. Studies on nutritional value of fermented products, have reported that fermentation increase the content of riboflavin, thiamine, niacin and ascorbic acid while improving the concentration of some amino acids (Jespersen, 2003). Further studies are required to elucidate the real impact of Btana transformation on date's nutritional value.

Strain's identification to the species level

Strains were tentatively designated to species level using common physiological and biochemical tests. Garvie (1986) established that production of dextran exclude many *Leuconostoc* species mainly: *L. lactis* and *L. citreum* but during the past 15 years, studies employing both phenotypic and genetic identification have lead to taxonomic revisions of the genus *Leuconostoc* (Justé *et al.*, 2008d). Ghazi *et al.* (2009) reported that *Leuconostoc* dextran (+) can be easily differentiated by the ability to ferment arabinose. By using Garvie's (1986) phenotypic identification scheme verified by ADN-ADN hybridization, AB_{04} and AB_{11}, are *Ln mesenteroides* subsp *dextranicum* according to the following carbohydrate features: Arabinose (-), galactose (d), lactose (d), mannitol (-), mannose (d), mellibiose (d), raffinose (+), mellibiose

(-), ribose (d), xylose(d), maltose (+), rhamnose (d), sorbitol (+). While MT_{03}, MT_{05} are assigned to *Ln mesenteroides* subsp *mesenteroides* based upon the following carbohydrate pattern: Arabinose (d), galactose (+), lactose (+), mannitol (-), mannose (+), raffinose (d), mellibiose (-), ribose (+), xylose (+), maltose (+, d), rhamnose (-), sorbitol (-). We notice that fermentation of mannitol, raffinose, mellibiose by AB_{04} and AB_{11} is delayed compared with Garvie's sheme. Variation in carbohydrates assimilation has been already explained by Ghazi *et al.* (2009) depending upon inoculums size and time of delayed reaction. In addition, Justé *et al.* (2008d) assume that bacterial adaptation to environmental conditions has the potential to alter the genome in such way that the organism develops new characters, which may explain the different behavior of the strains. However, the patterns obtained here are mostly closer to those of the described species. Physiological tests are very similar for all the studied strains. However, MT_{03} and MT_{05} undergo a weak growth at 37°C. It is well established that most species of *Leuconostoc's species* are Mesophillic, their growth temperature range from 10°C to 30°C.

Acidification and enzymatic activity

As presented in Table 5, low acidifying activity was recorded for the strains; they coagulate milk very slowly after a long-delayed acid formation time (after 4 days). This feature are being commonly observed in *Leuconostoc* sp, thus they are implied only in the earlier milk acidification stages in dairy product since few amount of lactic acid are produced (heterefermentative) and their lack of proteolytic activity (Barrangou *et al.*, 2002). So far, study of broth acidification by the isolated strains indicates a weak acidification power as it is recognized for the others lactic acid bacteria. pHm values ranged from 4.45 to 4.24 and were nearly identical for all strains. The time required for attain these values vary from 26 to 28 hour. These results are very close to those reported by Devoyod and Poullain (1988) and Stiles (1994) who achieved a final pH between 5 and 4, 4 in glucose broth inoculated with *Leuconostoc.* The highest value of Vm (0.57) was registered with MT_{05}, but the strain had the lowest acidifying activity may be due to the brutal decrease of pH. It was shown previously that *Leuconostoc mesenteroides*, acidify chou juice very weakly since it need 26 days to decrease the initial pH from 6.03 to 3.8 (Barrangou *et al.*, 2002). The main technological propriety of this bacterium relies only on flavor development therefore it is widely used for enhancement of organoleptic proprieties of fermented products (Sawale and Lele, 2010). This propriety needs to be investigated in the date product "Btana".

Growth study

As seen in Table 5 growth rate do not vary significantly between isolates, our results are within the range observed by Huang *et al.* (1994) ($\mu = 0.26$ $^{-}$h) for *Ln. mesenteroides* ssp. *mesenteroides.* But Bellengier *et al.* (1997) found a high value in MRS broth. This rate is more significant in milk (0, 63 $^{-}$h) according to the same authors. Dols *et al.* (1997a) proved that *Leuconostoc mesenteroides* displayed different growth rates depending on carbon source used. Subsequently, various yield were observed; 0, 58 $^{-}$h (glucose), 0, 59 $^{-}$h (fructose) 0, 60 $^{-}$h (fructose + glucose) and 0, 98 $^{-}$h (sucrose). Nunez and Medina (1979) established an average of (μ =0, 46 $^{-}$h) for 4 strains of *Ln. dextranicum.* As seen this value is very high than our result (0.17, 0.25 $^{-}$h).

In our case, the values of (μ) were obtained under small volume of medium MRS (10 ml) and under static conditions. Such factors explain the result obtained, and urge us to establish similar experimental conditions for an exhaustive comparison with other studies. In general, it is clear that sucrose enhance growth of *Leuconostoc* more than others carbon sources. It is possible that high concentration of this carbon source in date cultivar "Hmira" sustains growth of *Leuconostoc* strains in Btana. In other hand, the selected isolates have good osmotic tolerance which explains their unusual viability in 50% sucrose broth. This suggests that isolates are very adapted to osmotic pressure prevailing in Btana. In addition to this important propriety the isolates (AB_{04}, AB_{11}, MT_{05}) exhibited a broad antibacterial activity against the target bacteria. Oyetayo (2004) have studied the antagonistic potential of *Lactobacillus* sp. isolated from different sources; he reported a narrow inhibition zone (15 mm) with *Pseudomonas aeruginosa* NCIB 950 and (35 mm) with *Staphylococcus aureus* NCIB 8588. However it is known that *Leuconostoc* sp. exerted an antagonistic activity by a wide range of excreted metabolites like organic acids (thereby pH reduction is occurred), diacetyl, CO_2 and hydrogen peroxide (H_2O_2). In addition to these primary metabilites inhibitors, a number of authors have described bacteriocins (leucocin), excreted by *Leuconostoc* sp (Björkroth and Holzapfel, 2006; Xiraphi *et al.*, 2008). They are active against other LAB and closely related Gram positive bacteria. But here we revealed an inhibition toward Gram negative bacteria too (*E. coli*, *P. aeuroginosa*). The antibacterial activities of strains (AB_{04}, AB_{11}, MT_{05}) described in this study might suggest their contribution in biosafety of the

traditional date product Btana either by their primary metabolites or by bacteriocins.

Conclusion

This preliminary study indicates that the traditional date product "Btana" support growth of *Leuconostoc* sp. which represents an important group in lactic acid bacteria, responsible for some products qualities. Further investigations are needed to determine the role of this microbial group during Btana preservation. Other studies will clarify the biological process that undergo this traditional product to highlight the real interactions existed between factors including, chemical composition of Btana, and the dynamic of micro flora. By all, this study aimed to maintain and strengthen the cultural heritage and the how-know related to date transformation.

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