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Comparison of MALDI TOF MS profiling and 16S rRNA gene identification of presumptive lactic acid bacteria isolated from the traditional Algerian date product "Btana".

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Abstract – The present study sat the purpose to compare the accuracy of identification of 68 strains lactic acid bacteria originating from Btana samples (Bouchegroun, Sali, Algeria) Btana by means of 16s RNA sequencing and Matrix-assisted laser desorption ⁄ ioni-zation time-of-flight mass spectrometry (MALDI-TOFMS) MALDI TOF. Dilution of Btana samples were cultured on MRS, M17 and MSE media. For MALDI TOF identification, a cell extract was prepared from each isolates then coated with matrix of 1 µL of a 0.5% (w/v) alpha-cyano-4-hydroxycinnamic acid solution (α-CHCA). The best quality spectra was selected then compared against an in-house (LMG) built reference database. Five assigned cluster identified as Weissella paramesenteroides, Weissella cibaria/confuse, Leuconostoc citreum, Leuconostoc pseudomesenteroides, and Lactobacillus plantarum and one AAB species Gluconobacter cerinus. A representative strain of each cluster was selected for identification by 16S rRNA gene analysis. Sequences comparison with Genbank database confirmed accurately the strain's identity made already by MALDI-TOF. According to API 50 CHL essay, 7 carbohydrates (D-glucose, D-fructose, D-mannose, N-acetyl glucosamine, D-maltose, D-lactose, D-saccharose were systematically used by the five LAB strains. Which support that the identified species are adapted to the high sugar environment of Btana.

Keywords: Btana, date fruit, traditional food, lactic acid bacteria, high sugar food, MALDI-TOF MS.

1. Introduction

Exploration of the microbial diversity of traditional food and screening of microbial group with interesting proprieties urge laboratory team to apply simple, fast, cost effective, and reliable techniques to perform daily reproducible identification schemes (Welker, 2011). Till recently, researchers consider molecular techniques especially, DNA and sequencing-based tools like 16 s gene sequencing as a golden standard for bacterial identification that offer an accurate results (Gantzias et al., 2020; Ranjbar et al., 2014). However this technique needs highly sophisticated instrumentation, and much associated costs including sample preparation, expansive reagents that made the technique prohibitive for general use (Sauer et al., 2008). The present study aims at identifying lactic acid bacteria in the traditional date product Btana by means of 16s rRNA sequencing and Matrix-assisted laser desorption ⁄ ioni-zation time-of-flight mass spectrometry (MALDI-TOFMS) MALDI TOF (Cheng et al., 2018; Angelakis et al, 2011) . Recently microbiologists underscore the usefulness of this technique as a rapid and reliable method of microbial identification (Tanigawa et al., 2010. MALDI TOF technique is a bacterial chemotaxonomic method which can identify bacteria at the species and genus levels and has been proven to be effective. Moreover, it offers easiness in sample preparation and generates extraordinarily rapid data that can be simply acquired and analyzed (Fagerquist et al., 2010). The acquired data are then subjected to Library-Based comparison to determine the similarity rate with the reference species spectra implemented in the local or universal database. In this regard we attempt to apply this technique with presumptive lactic acid bacteria isolated from Btana. The product is basically fermented by a group of osmophilic yeasts as has been determined by our previous study (Abekhti et al, 2013). However, our plan work was inspired by the existence of some product that is co-fermented by yeasts and lactic acid bacteria and also by the spontaneous feature of the fermentation that might involve a mixture of microorganism (Lino et al., 2021). By far, there is no report in literature on the use of MALDI-TOF MS in identification of the LAB microflora of the traditional fermented date product Btana. Our current study aimed to explore this particular microflora, by using Maldi-TOF MS in parallel with 16S rRNA sequencing. This comparison may prove the usefulness of the proteomic technique to in the rapid identification of the lacic acid bacteria and their distribution in the fermented date product Btana.

4. Materials and methods

In order to isolate LAB from Btana, Five samples (each 250g) from Direct Btana method obtained from Bouchegroun (Biskra, Algeria) were blended together with a volume of TSI liquid medium Triple Sugar Iron (TSI) slants (M021I, HiMedia) at a ratio of 1/9 p/v in order to dissolute the present lactic acid microflora. The same treatment was performed with a Indirect Btana method samples collected from Sali (Adrar, Algeria). 1 ml of each dilution was cultured on MRS (Man Rogosa Sharpe, Biokar,. Beauvais, France). M17 (Biokar,. Beauvais, France) and MSE (Mayeux Sandine Elliker, Biokar,. Beauvais, France)

Man Sandin Eliker) medium.

Extract Sample preparation.

Presumptive LAB cultures were transferred to (laboratory of microbiology Gent LMG) in Belgium for identification by MALDI TOF technique. For that aim, colonies were cultivated aerobically at 28°C for 48 h on MRS for two consecutive generations (Wieme et al., 2014)..

A cell extract was prepared from each isolates according to Freiwald and Sauer (2009) for MALDI-TOF MS analysis. Briefly, one loop of the bacterial culture was harvested from single colony than suspended into 300 µL Milli-Q water. Next, 900 µL of absolute ethanol was added and the bacterial suspension was centrifuged for 3 min at 14,000 rpm.

After centrifugation and removal of the supernatant, cells were extracted with 50 µL 70% formic acid and 50 µL acetonitrile by vortexing for 1 min (Wieme et al., 2014; Sato et al., 2012). Then; residual cell constituents and intact cells were eliminated through centrifugation for 3 min at 14,000 rpm. The yielded supernatant was used for MALDI-TOF MS analysis. 1 µl of each supernatant was spotted on four wells of 384 stainless steel MALDI target plate (AB Sciex, The Netherlands) to obtain four replicates. After drying at room temperature, each well was overlaid with 1 µL of a 0.5% (w/v) alpha-cyano-4-hydroxycinnamic acid solution (α-CHCA) in 50:48:2 acetonitrile:water:trifluoroacetic water-solvent. Then the matrix was kept for dry before analysis.

MALDI-TOF MS analysis.

MALDI-TOF bacterial mass spectra, were obtained by 4800 Plus MALDI TOF / TOF Analyzer (Applied Biosystems, Framingham, MA, USA). The system was set in linear positive ion mode within the range of 2 -20 kDa. Molecule excitation was made by a 200-Hz-tripled UV Nd:YAG laser operating at a wavelength of 355 nm to generate ions which were subsequently accelerated in a 20-kV electric field through a grid at 19.2 kV and separated according to their m/z ratio in a 1.5 m long linear field-free drift region. For each spot, 40 sub-spectra each consisting of 50 laser shots at 50 random positions (2000 shots per well in total) within the spot were collected, automatically generating spectral mass profile in the range from 4200 and 5700 Da (Wieme et al, 2014a; Assamoi et al, 2016, Nguyen et al 2013).

Before sample analysis, a calibration step of the mass spectra was performed using the protein calibration standard I containing: cytochrome C, insulin, I, myoglobin and ubiquitin supplemented with the adrenocorticotropic hormone fragment 18–39 all acquired from Sigma-Aldrich. Bacterial Test Standard (Bruker Daltonics GmbH, Leipzig, Germany) was included as positive control for each

measurement. (Wieme et al, 2014a). Next, Raw data (mass spectra) were retrieved as t2d files from the 4800 plus MALDI TOF / TOF analyzer, then imported to the Data Explorer 4.0 software (Applied Maths) and transformed to text files. These text files were imported as input into the BioNumerics package and converted to fingerprints for further analysis (Gantziasa et al, 2020) . For each isolate, a best quality spectrum was selected based on peak intensity, background, and peak richness, then compared against an in-house (LMG) built reference database (Wieme et al., 2014a) by calculating the Pearson's product moment correlation coefficient. The data were subsequently converted into a binary character set and the spectra were clustered using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster algorithm (Wieme et al, 2014a).

Following MALDI TOF identification, a representative strain of each cluster was selected for reliable identification by 16S rRNA gene sequencing in food department at Liege University (Belgium).

The template DNA was extracted and amplified in 20 µl volumes each containing: 2 µl extracted DNA, 2 µl of dNTPs (2mM), 1.6 µl of MgCl2 (25 mM), 2 µl PCR buffer (10×), 0.8 µl of each primer (10 µM) 16S-1500 F: 5'-GAG- TTT-GAT-CMT-GGC-TCA-G-3' (Eurogentec, 3968107, Tm=60°C) 16S-1500 REV: 5'-TAC-GGT-TAC-CTT-GTT-ACG-AC-3' (Eurogentec, 3968108 Tm=58 °C.) and 0.2 µl of Taq DNA polymerase (2.5U, Fastastart, Roch, Germany). The PCR program was performed according to Abekhti et al (2017) with a Mastercycler gradient (Eppendorf, Westbury, NY). DNA amplicons of the distinct isolates were purified then transferred to GIGA centre (Liège, Belgium) for sequencing. Sequences of rRNA gene were edited and the identities of isolates were obtained using the online BLAST program implemented in the GenBank public database.

Carbohydrate fermentation of the LAB isolates:

In order to determine the metabolic profile of the identified LAB, a carbohydrate fermentation test was carried out using API 50 CHL strips (BioMerieux, , France) according to the manufacturer's recommendations.

5. Tables and figures

Tables and figures must be included in the text and completed with titles, units, and other information necessary to understand the table or figure without referring to the text.

Tables should be kept to a minimum.
The unit of measurement used in a table should be stated.
Tables should be numbered consecutively.

Figures/Graphics should be prepared in GIF, TIFF, JPEG or PowerPoint before including in the definitive manuscript.
Tables and Figures should be appropriately cited in the manuscript.

6. Results

Following incubation, 68 isolates were randomly picked up from MRS (36 isolates), MSE (15 isolates) and M17 (17 isolates) for preliminary identification purpose. The majority of the isolates were selected from the DBM samples (Boucgegroun) while 11 isolates were selected from UBM (Sali)

Either catalase positive or Gram negative isolates were ignored in subsequent analysis. Some other isolates did not grow on MRS medium in LMG laboratory and hence were subtracted from analysis.

Preliminary identification was conducted to cluster isolates in bacterial group according to their phenotypical characters. Regarding MALDI TOF identification, good quality spectra were obtained for all isolates a cut-off was calculated to delineate clusters based on quadruplicate analysis of the same 33 isolates (Fig 1). Mean similarities ranged from 90.63% to 99.80%.

Analysis against the local database of LMG bacteria culture collection revealed the presence of 5 collective clusters (composed of 2—12 strains), and 7 individual clusters (Fig 2). In matter of the MALDI TOF spectra, although, there are little differences, the global topology of the MALDI-TOF MS spectra was similar for most strains sharing the same cluster (Fig 1).

Most of examined strains (33) present species specific clusters except for (R-48932 (M17Z02), R-48933 (M17Z04), R-48937 (M17Z01) which represent a clone of the same species that occupied unique position within the dendrogram and remain unidentified. Seemingly, the unique strain cluster R-48902 (BOSO) couldn't be assigned to any species in the database. In short, MALDI TOF result indicate the presence of a very limited number of different LAB clones regrouped in five assigned cluster identified as (*Weissella parameseneroides, Weissella cibaria/confusa, Leuconostoc citreum, Leuconostoc pseudomesenteroides,* and *Lactobacillus plantarum*) and one AAB species (*Gluconobacter cerinus*).

Overall, the first cluster is composed of strains that have the same MALDI TOF pattern as the non type LMG strain '*Leuconostoc pseudomesenteroides* LMG 18969 with a good correlation value (86.3). Additionally, according to the correlation value between isolates and their common source (Bouchegroun), we might say they represent clones of the same species at least for the three strains (R-48911(MRSBO05),R-48915 (MRSBO0Q), R-48952 (MRSBQ).

On the other hand, the second cluster regroup six isolates all from Sali BTana and divided into two subgroups, both matched with The type strain *Weissella paramesenteroides* LMG 9852 T. the first subgroup encompasses five strains matching *Weissella paramesenteroides* LMG 9852 T with 60.9 whereas the second subgroup is represented by a unique strain (R-48922 (MRSZ9) by a correlation value of 62.3. Indeed pick profile of the two subgroups is barely the same with some differences (Fig. 1). The strain R-48922 (MRSZ9) has 23 picks with 6 principal pick. While MRSZ07, MRSZ02, MRSZ1, MRSZ3, from Sali Btana have 36 pick composed of 10 major picks.

Regarding the third cluster, it encompasses a unique strain matching highly (85.5) with the LMG type strain *Gluconobacter cerinus* LMG 1368 T. The fourth cluster is composed of 14 strains shared by two clusters with a very close correlation value 95.2. Five strains were isolated from M17, eight strains (9) from MRS medium, whereas one strain was isolated from MSE medium. MALDI-TOF MS analysis clearly defined these strains as *Weissella cibaria/confuse* with 65.9 Pearson correlation as both species present similar MALDI-TOF MS pattern. The strains in both clusters might represent a clone of two strains awing to the high observed correlation (97-99).

The fifth cluster is formed of two strains presenting high correlation 90.1 with *Leuconostoc citreum* LMG 23114. The last cluster is composed of a unique strain assigned to the LMG type strain *Lactobacillus plantarum* LMG 6907 T. From each cluster, a representative strain was selected randomly for 16S rRNA gene sequencing and carbohydrate utilization test using API 50CH. The selected representative strains are shown in the reduced dendrogram represented in Fig 3. 16S rRNA gene sequencing confirmed accurately the strain's identification previously made by MALDI-TOF. Furthermore, this molecular technique has resolved the ambiguity stated with MALDI-TOF technique regarding the assignation of the fifth cluster to either *W. confusa* or *W. cibaria* . 16S rRNA gene sequencing has assigned the cluster to *W. confusa* with the maximum correlating score. On the other hand, it was found that out of the 49 substrates tested by API 50 CHL, 22 substrates were assimilated either by one or more strains. Considering all the carbon sources, only 7 carbohydrates (D-glucose, D-fructose, D-mannose, N-acetyl glucosamine, D-maltose, D-lactose, D-saccharose were systematically used by the five LAB strains. Whereas 20 sugars were not used by all the studied species namely : Glycerol, Erythritol, D-arabinose, L-xylose, D-adonitel, Methyl-D-xylopyranoside, L-sorbose, L-rhamnose,

Dulcitol, Inositol , inulin, Amidon,Glycogen, Xylitol, D-Lyxose, D-Tagatose, D-Fucose, L-Fucose, D-arabitol, L-arabitol, Pottasium ketogluconate. By far, Methyl-αD-glucopyranoside (21) was metabolized by *Leuconostoc* species only. Among the carbon sources distinguishing strains from each other, we mainly noticed that some species could not metabolize some particular sugars; L citreum (D-ribose, D-galactose), *W paramesenteroides* (Arbutine, Salicin, D-Cellibiose, Gentibiose), *W confusa* (D-Trehalose). On the other hand, there are some sugars which are metabolized by only one of the species under investigation ; Methyl-αD-mannopyranoside (20) and Esculine by *Leuconostoc pseudomesenteroides*; D-Melezitose by *L plantarum* and Pottasium ketogluconate (48) by *L. pseudomesenteroides.*

7. Discussion

During this study, we have sat the purpose to compare the accuracy of the identity of 33 strains isolated from LAB medium (MRS, M17, MSE) by using two different methods; MALDI TOF and 16S rRNA gene sequencing . 16S rRNA sequencing has a wide use in laboratory, but despite its performance, it is still not affordable at a daily use due to reagents and labor costs (Sánchez-Juanes et al., 2020). Also, its turnaround time take at least two days between isolate culturing, DNA extraction, PCR, and sequencing. These costs limit reproduction and so reduce the description of a big number of isolates (Pomastowski, 2019). Currently MALDI-TOF is getting more popular due to its versatile advantages and ease of use (Rabodoarivelo et al., 2016). The cost of reagents is cheaper than molecular and biochemical phenotyping methods. At this regard, it offers the possibility in doing protein extraction more than one time (duplicate, triplicate) on sample and pure isolates (Pomastowski, 2019).

Our data reveal that MALDI TOF MS correctly identifies all of the LAB isolates in total accordance with 16S rRNA gene sequencing. This is a reason to consider this technique routinely. Nevertheless, there are some strains that remain unidentified with this technique, because of the gap in the reference spectra implemented in the MALDI database. This is frequently encountered with bacteria inhabiting particular environmental which are more diverse implicating difficulties in their identification. On this regard, database enrichment and refinement are key elements for the generalization of MALDI-TOF MS tool as a powerful and reliable identification method. According to our results, and despite the fact that Btana is a sugar rich environment, we have detected five LAB that survived this harsh

physiochemical conditions. Many reports revealed the ability of LAB to tolerate extraordinary habitats due to their resistance to chaotropic solutes like glycerol, fructose and hydrophobic stressors (Lievens et al. 2015, Franz and Holzapfel 2011). The identified species are more often ubiquitous usually present in rich sugar environment and some of them are regularly isolated from fermented fruits and are involved in development of specific flavors and distinguishable sensory characteristics (Garcia et al 2019).

Our findings regarding sugar consumption test, confirms the ability of all isolated species to use sugars found in Btana (sucrose, glucose ad fructose). API 50 CH essay (Table 01) revealed the ability of the LAB strains to metabolize a limited number of the carbohydrate sources (17-23 sources). However they have the ability to utilize multiple fruit sugars (sucrose, glucose, fructose, maltose). It is obvious that the selected species are osmoolerant which means that they are able to grow at 50% glucose or even in the presence of 60% glucose. Abekhti et al, (2014) reported that Btana is a rich sugar medium that offers an appreciable capability of osmotolerant species to overcome osmotic stress. The origin of the LAB microbiota present in Btana is water, air, soil, and other environmental sources. According to our results (Table 1), Weissella is the most detected genera in Btana samples. It is represented by two species (*W. paramesenteroides* and *W. confusa*) accounting for twenty isolates shared by the direct Btana method (Sali) and Undirect Btana method (Bouchegroun). These species are frequently encountered in high sugar environment (Di Cagno et al, 2016). *Weissella confusa* was the most abundant species, present in both btana types. However the majority of the strains were isolated from Bouchegroun samples. It was isolated from all the media (MRS, M17, MSE) which can justifies its widespread in this product. It is a new finding, as far as our previous observation revealed the dominance of *L. mesentoroides* (Abekhti et al., 2014). Several hypothesis can be made face of this discrepancy, one of them is that *W. confusa* was originally classified as *Leuconostoc* and there is a limited biochemical features that can distinguish the two species (Schillinger et al, 2008). *Weissella confusa* strains have been detected as a natural occurring microbiota in sugar cane, carrot juice and occasionally in raw milk and sewage (Fusco et al, 2015). Regarding, W. paramesenteroides, many investigation reported its presence in a variety of fermented fruits and vegetables (Chen et al., 2013b), and even from raw fruit like banana fruit (Chen et al., 2017). In some reports, it has been mentioned that this LAB is more often associated with

fermented foods rather than fresh fruits and vegetables (Fessard and Remize, 2019) and it has recently attracted attention due to their high dextran production ability (Kamboj et al., 2015). Also, the presence of *Leuconostoc* in the product is not surprising owing to the high osmotic pressure of Btana which is selective to this group of microorganisms. The current finding confirms our previous observation that *Leuconostoc* is a common species in the traditional date product Btana (Abekhti et al., 2014). Among the identified species; *L citreum* is getting a particular interest in biotechnological area, to produce functional oligosaccharides and dextran. Additionally it has an interesting enzymatic feature of catalyzing the synthesis of low molecular weight oligosaccharides from sucrose that are suggested as prebiotics in both food and cosmetic applications (Olivares-Illana et al, 2002).

By far, *L. plantarum* was frequently found in high sugar medium and often described as Fructophilic lactic acid bacteria that has the ability to colonize niches rich of high sugar (fructose)concentrations (Endo and Okada 2008). Moreover, it has been also detected in nectar and fruits (Endo et al. 2009). As such, dates and particularly Btana product, are suitable for the growth of *L. plantarum*, due to the presence of high proportion fructose according to (Abekhti et al 2021). Furthermore, *Gluconobacter cerinus* was the unique acetic acid bacteria found in our strain collection, it was surprisingly detected on M17 medium and was successfully subcultured on MRS medium. The species is a commonly detected in sugar-rich habitats like flowers and fruits and is able to grow in highly concentrated sugar solution (Sievers & Swings, 2005). Recently it gains importance in the agroecosystem for its ability to increase plant biomass. It might be one of the acetic acid bacteria responsible of the conversion of date juice to vinegar as has been reported by Matloob (2014).

8. Conclusion

Considering the direct Btana method used in preparation of Bouchegroun Btana, an original diversity could be preserved as far as the technique do not use any washing step that reduces the initial load of microorganism associated with date fruit. By contrary, few LAB isolates were isolated from Sali direct Btana method. Many washing steps are followed during preparation of direct btana method leading to a drastic lost in fruit associated microbiota, including eventually lactic acid bacteria. On the other hand, our study confirms the total correlation of MALDI TOF identification of Lactic acid bacteria isolated from Btana product with the reference molecular technique (16S rRNA gene sequencing). It is an affordable alternative offering rapid turnaround time for bacterial fingerprinting. It also, helps in avoiding unworthy identification of clone of the same strain. Moreover, since the use of MALDI OF, skills have made spectacular strides in the current decade in

terms of price per reaction and protocol handiness. We should seek creating a reference local library of the microbiota of the local traditional product, because Screening of the lactic acid bacteria adapted to Btana product characteristics will help selection of interesting group with applied properties like osmotic pressure resistance. Moreover, substantial efforts are required to standardize the MALDI TOF preparation protocols to improve reproducibility and establishing comparative studies. Database refinement and enrichment is a substantial element to enlarge the identification scheme spectra and consequetly overcoming the handicap of unidentified isolates. It is these easy-to-use methods that will prevail over state-of-the art and conventional biochemical essays at least in short budget laboratories.

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10. Tables and figures

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

The unit of measurement used in a table should be stated. Figures/Graphics should be prepared in GIF, TIFF, JPEG or PowerPoint before including in the definitive manuscript.
Tables and Figures should be appropriately cited in the manuscript.

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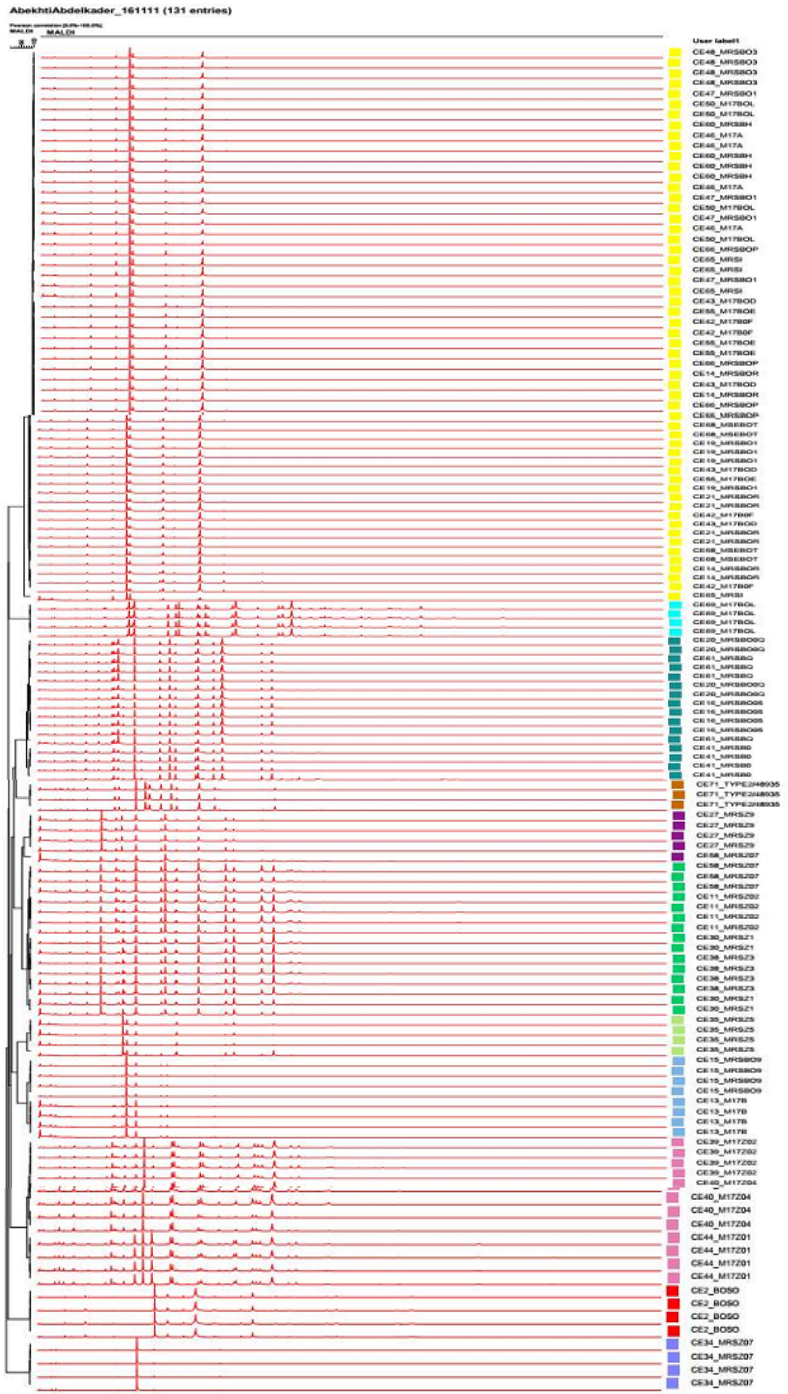

Fig 1. Quadruplicate spectra of the presumptive LAB from Btana product obtained by MALDI TOF spectral mass profiling in ge from 4200 and 5700 Da trough excitation by a 200-Hz-tripled UV Nd:YAG laser

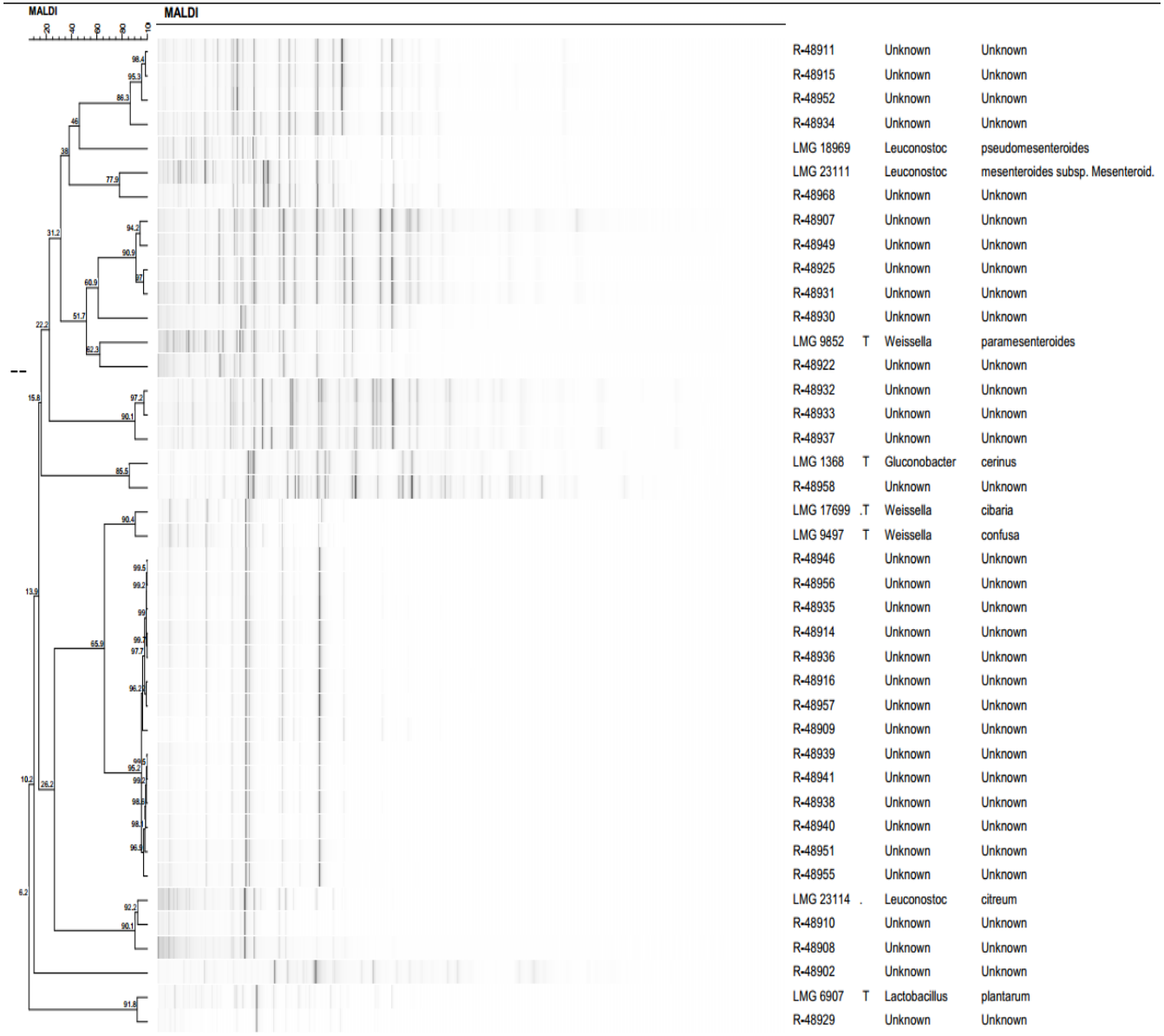

Fig 2. MALDI TOF dendrogram of presumptive LAB strains against LMG MALDI TOF database --

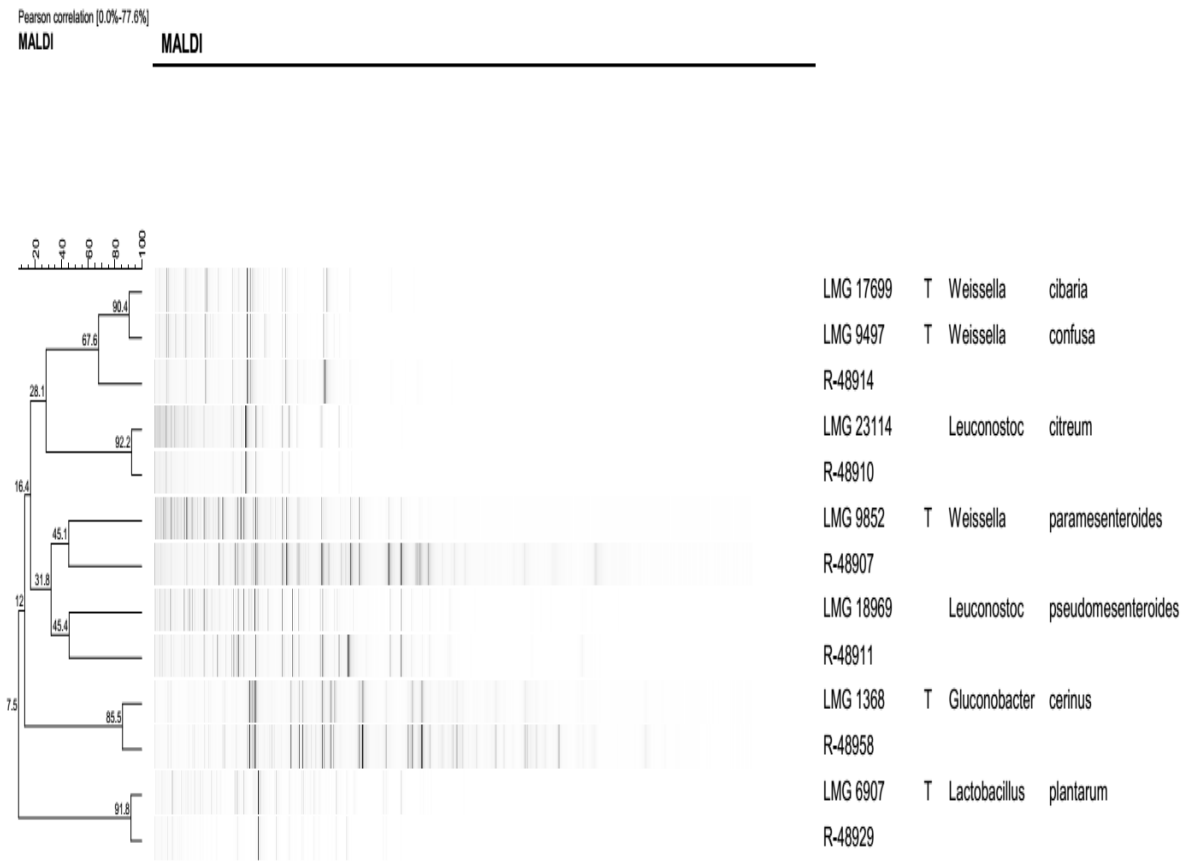

Fig 3 MALDI TOF dendrogram of representative strains selected for 16s rRNA sequencing and carbohydrate test (API 50CH for LAB)

Table 1. representing the identified clusters after MALDI TOF MS profiling and 16S rRNA gene sequencing of the 33 LAB isolated from Btana samples

Research code	Strain code, medium, origin	Correlation	MALDI TOF assignation	Sugar metabolized by the representative strain (in bold)	16S rRNA gene sequencing score
R-48911 R-48915 R-48952 R-48934 R-48968 (2)	MRSBO05, MRS, Bouchegroun MRSBO0Q, MRS, Bouchegroun MRSBQ, MRS, Bouchegroun MRSB0, MRS, Bouchegroun M17B0F, M17, Bouchegroun	86.3	Leuconostoc pseudomesenteroides LMG 18969	D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-sorbitol, Methyl-αD-mannopyranoside, Methyl-αD-glucopyranoside, N-acetylglucosamine, Arbutine, Esculine, Salicin, D-Cellibiose, D-Maltose, D-lactose,D-Melibiose, D-Sacharose, D-Trehalose, Raffinose, Gentibiose, Pottasium ketogluconate	L. pseudomesenteroides
R-48907 R-48949 R-48925 R-48931 R-48930	MRSZ02, MRS, Sali MRSZ07 , MRS, Sali MRSZ1, MRS, Sali MRSZ3, MRS, Sali MRSZ1, MRS, Sali	60.9	Weissella paramesenteroides LMG 9852 T	L-arabinose , D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, N-acetylglucosamine, D-Maltose, D-lactose, D-Melibiose, D-Sacharose, D-Trehalose, Raffinose, Potasium gluconate	W. paramesenteroides
R-48922	MRSZ9, MRS, Sali	62.3			
R-48958 R-48946 R-48956 R-48935 **R-48914** R-48936 R-48916 R-48957 R-48909	M17BOL M17, Bouchegroun M17BOE M17, Bouchegroun MRSBOP MRS, Bouchegroun M17B0F, M17, Bouchegroun MRSBO1, MRS, Bouchegroun M17BOD, MRS, Bouchegroun MRSBOR, MRS, Bouchegroun MSEBOT , MRS, Bouchegroun MRSBOR, MRS, Bouchegroun	85.5 65.9	Gluconobacter cerinus LMG 1368 T *W. confusa LMG 9497 T* *Weissella cibaria LMG 17699*		G. cerinus *W. confusa*
R-48939 R-48941 R-48938 R-48940 R-48951 R-48955	MRSBO1, MRS, Bouchegroun M17BOL , M17, Bouchegroun M17A, M17, Sali MRSBO3, MRS, Bouchegroun MRSBH, MRS, Bouchegroun MRSI, MRS, Sali			L-arabinose , D-ribose , D-xylose, D-galactose, D-glucose , D-fructose, D-mannose, N-acetylglucosamine, Amygdaline, Arbutine, Salicin, D-Cellibiose, D-Maltose, D-lactose, D-Sacharose, Gentibiose, Potasium gluconate	
R-48910 R-48908	MRSBO9, MRS, Bouchegroun M17B, M17, Bouchegroun	90.1	L. citreum LMG 23114	L-arabinose, D-glucose, D-fructose, D-mannose, D-mannitol, Methyl-αD-glucopyranoside, N-acetylglucosamine, Amygdaline, Arbutine, Salicin, D-Cellibiose, D-Maltose, D-lactose, D-Sacharose, D-Trehalose, Gentibiose, D-Turanose, Potasium gluconate	L. citreum

R-48929	MRS Z07, MRS, Sali	91.8	Lactobacillus plantarum LMG 6907 T	L-arabinose D-ribose, D-galactose, D-glucose , D-fructose, D-mannose, D-mannitol, D-sorbitol, N-acetylglucosamine, Amygdaline, Arbutine, Salicin, D-Cellibiose, D-Maltose, D-lactoseD-Melibiose, D-Sacharose, D-Trehalose, D-Melezitose, Raffinose, Gentibiose, D-Turanose, Potasium gluconate	Lactobacillus plantarum
R-48902	BOSO, MRS, Bouchegroun	/	unidentified	/	/
R-48932 R-48933 R-48937	M17Z02 , M17, Sali M17Z04 , M17, Sali M17Z01, M17, Sali	/	Unidentified unidentified unidentified	/	/