Trop J Nat Prod Res, June 2024; 8(6):7466-7473 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Isolation, Carbohydrate Metabolism Profile, and Molecular Identification of Plant-Derived Lactic Acid Bacteria from *Musa acuminata x balbisiana* and *Medinilla cummingii* Naudin

Kristina Casandra L. Pava[1]*, Glenda Z. Doblas[1,2], Joliesa Mae S. Toledo[1,2], Reggie Y. Dela Cruz[1,2]

[1]*Molecular Biology and Biotechnology, Genetics, and Microbiology Division, Institute of Biological Sciences, College of Arts and Sciences, Central Mindanao University, Musuan, Maramag, Bukidnon, 8710*
[2]*Tuklas Lunas Development Center, Central Mindanao University, Musuan, Maramag, Bukidnon, 8710*

ARTICLE INFO

Article history:
Received: 16 April 2024
Revised: 18 May 2024
Accepted: 29 May 2024
Published online 01 July 2024

ABSTRACT

Plants serve as a conducive milieu for diverse microorganisms, including lactic acid bacteria (LAB). Heightened concerns surrounding issues related to dairy products have catalyzed interest in exploring plant-derived LAB as valuable reservoirs for innovative strains with applications in both industrial and medical domains. The regional variability of LAB species and the lack of documented isolation studies in Mindanao, Philippines, especially concerning endemic flowers, are noteworthy factors. Consequently, the primary aim of this study is to systematically isolate, purify, characterize, and identify LAB originating from two plant sources: *Musa acuminata x balbisiana* and *Medinilla cummingii* Naudin. The two LAB isolates denoted as BNA1 and MED1, respectively, manifest the cardinal attributes indicative of lactic acid bacteria, namely gram-positive, catalase-negative, coccobacilli in morphology, and display proficient clearing around the colony through lactic acid production on MRS agar media supplemented with 1% calcium carbonate ($CaCO_3$). Using the API 50 CHL system to study phenotypic characteristics through sugar fermentation showed that BNA1 has vigorous fermentation activity on 27 carbohydrates and weak fermentation activity on 14 carbohydrates. Meanwhile, MED1 has demonstrated robust fermentation activity on 21 carbohydrates. Based on the 16S rRNA gene, BNA1 and MED1 are identified as MED1 as *Lactiplantibacillus* sp. (GenBank Accession Number PP627039) and *Enterococcus faecium* (GenBank Accession Number PP627037), respectively. The findings of this research will serve as a fundamental basis for future investigations, particularly on its potential as new probiotic strains from plants and their corresponding applications.
.

Keywords: *lactic acid bacteria, plant-derived, carbohydrate profiling, probiotics.*

Introduction

Lactic acid bacteria (LAB) constitute a Gram-positive, catalase-negative, and non-sporulating bacterial group naturally occurring in diverse environments, including water, soil, plants, dairy products, fermented vegetables, gastrointestinal tract (GIT) of humans and animals.[1] LAB has an established historical application as probiotics2, representing a varied spectrum of genera.[2] The FAO/WHO defined *probiotics* as "living microorganisms that, when administered in sufficient quantities, provide a health benefit to the host" and deemed them safe with a "generally recognized as safe" (GRAS) designation.[3] Researchers attribute the inclusion of LAB in this category to its natural preservative qualities and its ability to impede the proliferation of harmful bacteria in food.[4]
The pursuit of novel probiotic strains has intensified as researchers seek diverse and unconventional sources to enhance the repertoire of health-promoting microorganisms.

*Correspondence Email: s.pava.kristinacasandra@cmu.edu.ph
Tel: +639173032472
.

Citation: Pava KCL, Doblas GZ, Toledo JMS, Dela Cruz RY. Isolation, Carbohydrate Metabolism Profile, and Molecular Identification of Plant-Derived Lactic Acid Bacteria from *Musa acuminata x balbisiana* and *Medinilla cummingii* Naudin. Trop J Nat Prod Res. 2024; 8(6): 7466-7473. https://doi.org/10.26538/tjnpr/v8i6.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The unique attributes of plants, characterized by a high carbohydrate but low protein content and a slightly acidic pH, render them an ideal environment for various microorganisms, including LAB.[4-6] Plant-derived LAB exhibits various metabolic and functional properties, encompassing vitamins, minerals, antioxidants, phenolics, and dietary fibers.[7] The reported species diversity of LAB found on fruits and flowers includes genera such as *Lactobacillus, Lactiplantibacillus, Lactococcus, Leuconostoc, Fructobacillus, Lactobacillus, Enterococcus, Pediococcus, Streptococcus*, and *Weissella.*[8]
Its status characterizes the Philippines as a mega-diverse country with high endemism of plants with medicinal and nutritional values.[9] The 'saba' banana (*Musa acuminata x balbisiana*, BBB Group) is the primary banana variety cultivated in the Philippines, making a substantial contribution to the local economy through extensive cultivation and processing into chips or crackers.[10-11] Moreover, banana inflorescences have been demonstrated to offer various health benefits, including antioxidant properties and potential therapeutic effects such as antidiabetic, anticancer, and cardioprotective properties.[12-13] Species of the genus *Medinilla* Gaudich are found throughout the Philippines and are cultivated primarily for their ornamental value.[14] Several *Medinilla* species have undergone analysis for active phytochemical compounds antimicrobial, and antifungal properties prior to their use as traditional medicine in various Asian countries.[15-16]
However, current scientific literature has given scant attention to the utilization of floral components of plants as an unconventional reservoir of LAB despite considerable research characterizing LAB

from traditional sources such as dairy products, fermented foods, and animal intestinal tracts. This study marks a substantial departure from conventional research paradigms by exploring endemic flowers in the country as an alternative reservoir for probiotic bacteria. The primary focus of this study is to isolate lactic acid bacteria (LAB) from the inflorescences of *Musa acuminata x balbisiana* and *Medinilla cummingii* Naudin, characterize their colony morphology, assess their carbohydrate fermentation profile using the API 50CHL system, identify the isolates through 16S rRNA gene sequencing, and perform phylogenetic analysis.

Materials and Methods

Isolation, screening, and purification of plant-derived LAB

Inflorescence from *Musa acuminata x balbisiana* was collected on January 2024 at a private banana farm in Brgy. Panadtalan, Maramag, Bukidnon, Philippines (7.81259° N, 125.01134° E), and propagated *Medinilla cummingii* Naudin was collected on April 2023 at a residential household at Musuan, Maramag, Bukidnon, Philippines (7°51'36.4"N 125°02'42.3" E), stored in sterile resealable bags, and transported to the laboratory in an icebox for analysis. stored in sterile resealable bags, and transported to the laboratory in an icebox for analysis. The inflorescence was dissected to obtain the florets, leaving the pistil and stamen intact. The samples were then enriched in MRS broth for 48 hours at 37°C following homogenization with a vortex. After enrichment, the samples were serially diluted and plated on MRS agar (Merck Millipore, Germany) supplemented with 1% calcium carbonate ($CaCO_3$) (Scharlab, Spain) to stimulate LAB growth[17]. The plated samples were incubated at 37°C for 48 hours under aerobic conditions (Thermostatic Shaking Incubator, MF-I103B, MedFuture, China). Colonies exhibiting acid production by clearing around the colony were selected, purified on MRS agar plates using the streak plate method, and further characterized to obtain pure isolates[9].

Cultural, morphological and catalase evaluation

LAB screening was conducted employing the presumptive methodology outlined in a previous study.[8] Following three consecutive passages of the LAB isolate to achieve purification, discrete colonies were sub-cultured and provisionally identified based on cell morphology, cultural characteristics, Gram reaction (Medic Diagnostic Reagents, Philippines), and catalase test.

Carbohydrate fermentation profiling using API 50 CHL system

API 50 CH strips (API systems, BioMérieux, France) were used to characterize the carbohydrate fermentation profile of the isolates, following the manufacturer's instructions. The inoculation strips were incubated at 37°C for 48 h under aerobic conditions (Thermostatic Shaking Incubator, MF-I103B, MedFuture, China) before the reactions were evaluated. After a 24 h and 48 h incubation period at 37°C, results were obtained by observation of a color change in the indicator bromocresol purple from dark purple to yellow or greenish, which indicates the carbohydrate fermentation activity of the tested isolates.[18]

Molecular identification of the LAB isolates using 16S rRNA gene

Glycerol stocks of LAB isolates were forwarded to Macrogen, Inc., Seoul, South Korea, subjected to DNA sequencing (ABI PRISM 3730XL Analyzer, Applied Biosystems) using primers that amplify the DNA region spanning positions 27F (5'AGAGTTTGATCCTGGCTCAG-3') to 1492R (5'-TACGGTACCTTGTTACGACTT-3') of bacterial 16S rRNA genes. The. ab1 forward and reverse reads were submitted to the Philippine Genome Center Visayas Satellite Facility for bioinformatics analysis. The quality of sequences was assessed, and low-quality base pairs were trimmed using PreGap4 (Staden Package Program, Medical Research Council (MRC) Laboratory of Molecular Biology, United Kingdom). Consensus sequences were generated using the Gap4 shotgun assembly approach. Homologous sequences were determined by submitting the consensus sequences to the National Center for Biotechnology Information (NCBI) BLASTN tool. The sequences, along with their BLASTN matches, were downloaded. The evolutionary relationships among the sequences were elucidated by constructing phylogenetic trees in MEGA 11 (MEGA Software Team, MEGA 11, 2021) using the Maximum- Likelihood method. The sequences were aligned using ClustalW of the same program, and the Model option identified the Kimura 2-parameter model (K2) and the Kimura 2-parameter model with Gamma distribution (K2+G) as the most suitable fit for BNA1 and MED1 isolates, respectively. An outgroup was selected for each sample to provide a reference point in the analysis

Results and Discussion

Cultural, morphological and catalase evaluation of plant-derived LAB

Twelve (12) colonies from *Musa acuminata x balbisiana* and six (6) from *Medinilla cummingii* Naudin were randomly selected from the colonies exhibiting large clear zones around the colonies. One (1) LAB isolate from each plant sample with distinct morphological characteristics was chosen based on observation of colonies on MRS agar and labelled as BNA1 (from *Musa acuminata* × *balbisiana*) and MED1 (from *Medinilla cummingii*), respectively. Table 1 summarizes the cultural, morphological and catalase evaluation of LAB isolates. BNA1is a gram-positive, catalase-negative coccobacillus (Figure 1a). It exhibits a creamy-yellow circular colony with a mucoid or glistening convex elevation on MRS agar. MED1 is gram-positive, catalase-negative cocci (Figure 1b) and formed creamy-white circular colonies on MRS agar with a mucoid or glistening convex elevation (Table 1).

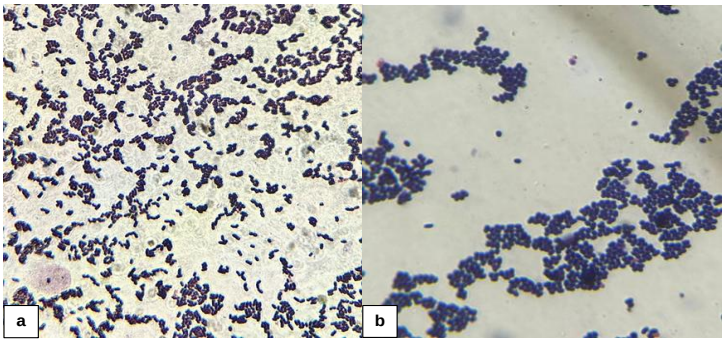

Figure 1: LAB Cell Morphology (1000X) of a) BNA1 exhibiting a Gram-positive coccobacilli morphology; and b) MED1 exhibiting a Gram-positive cocci morphology

Table 1: Summary of cultural, morphological and catalase evaluation of LAB isolates.

General Characteristics	LAB isolates	
	BNA1	**MED1**
Growth pattern in MRS Broth	Turbid	Turbid
Colony morphology in MRS Agar	Mucoid/Glistening, Smooth, Round, Entire Margin, Convex colony	Mucoid/Glistening, Smooth, Round, Entire Margin, Convex Colony
Colony color in MRS agar	Creamy-yellowish colonies	Creamy-white colonies
Gram Reaction	Gram-positive, rod	Gram-positive, cocci
Growth Conditions	37°C, Aerobic	37°C, Aerobic
Catalase test	Negative	Negative

BNA1 was isolated from Saba (*Musa acuminata x balbisiana*), a predominantly banana variety cultivated in the Philippines.

Banana flowers or banana blossoms have been demonstrated to possess a range of biological activities that offer potential health benefits, including notable antioxidant properties.[19] Several studies[20-22] have also examined banana flowers' nutrient content, physical and chemical properties, and functional properties. These studies show that they have a lot of dietary fibers (70% or 5.74/100g), carbohydrates (53.78% or 9.9/100g), and protein (19.60% or 1.6/100g). Dietary fibers include most soluble dietary fibers (SDFs) like fructo-oligosaccharides, galacto-oligosaccharides, inulin, and β-glucan, to name a few, and have positive effects on the proliferation of probiotics in the gut.[23] Moreover, studies have shown that the dietary fibers found in banana flowers alleviate constipation and promote gastrointestinal well-being due to their prebiotic properties, which act as indigestible food constituents. These properties favorably impact the host organism by selectively fostering the proliferation and functionality of specific bacteria within the colon, thereby enhancing overall host health.[22, 24]

MED1 was isolated from *M. cummingii*, a species of flowering plants in the family Melastomataceae, consisting of around 400 species of tropical plants native to Southeast Asia, the Pacific islands, and South America.[25] The diversity of the *Medinilla* genus is prominent in the Philippines and is commonly found in lowland rainforests and montane forests, with some species also occurring in mossy forests and ultramafic forest.[26-27] Several species of *Medinilla* have been used as traditional medicine in various Asian countries and were tested for active phytochemical compounds with antimicrobial and antifungal properties[28-29]. There are currently no available data on published journal articles regarding the presence of LAB in *Medinilla* species found in the Philippines. However, the Philippines has various lactic acid-fermented indigenous food products, which have different characteristics depending on the region, materials used, and process employed.[30]

The LAB isolates, BNA1 and MED1, displayed characteristics typical of lactic acid bacteria. Typical LAB are Gram-positive due to a thick peptidoglycan layer in their cell walls.[31] The absence of catalase activity in LAB is linked to their metabolism, as these bacteria predominantly ferment carbohydrates to produce lactic acid, which creates an acidic environment.[32]

Carbohydrate fermentation profile using API 50 CHL system

Table 2 summarizes the carbohydrate fermentation profiles of the isolates. BNA1 demonstrated robust utilization of 26 carbohydrates, resulting in a color change of the bromocresol purple indicator from purple to yellow (except for well 26 in the esculin hydrolysis test), while exhibiting weaker utilization of 14 carbohydrates, leading to a color change from purple to green (Figure 2a). BNA1 exhibited robust metabolic activity towards a spectrum of 21 monosaccharides and their derivatives, encompassing D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhaminose, α-methyl-D-mannopyranoside, α-methyl-D-gluopyranoside, N-acetyl-glucosamine, and gluconate. Additionally, it displayed metabolic proficiency towards 4 disaccharides, namely D-cellobiose, D-maltose, D-lactose, and sucrose; 3 polysaccharides, like esculin ferric citrate, D-trehalose, melezitose, and D-turanose; and sugar alcohols such as glycerol, D-mannitol, D-sorbitol, amygdalin, salicin, gentibulose, and 5-keto-gluconate. However, BNA1 manifested weak fermentative capacity, as evidenced by the conversion of the indicator bromocresol purple to green when exposed to 7 monosaccharides, namely β-methyl-D-xyloside, D-xylose, D-tagatose, D-fucose, L-fucose, D-arabitol, and L-arabitol; 1 disaccharide, D-melibiose; 3 polysaccharides like starch, glycogen, and D-turanose; and 3 sugar alcohols, adonitol, dulcitol, and inositol.

MED1 exhibited vigorous utilization of 21 carbohydrates, causing the bromocresol purple indicator to transition from purple to yellow (Figure 2b). In addition, MED1 demonstrated vigorous metabolic proficiency toward 8 monosaccharides such as L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, α-methyl-D-mannopyranoside, and N-acetyl-glucosamine; 5 disaccharides, namely D-cellobiose, D-maltose, D-lactose, D-melibiose, and sucrose; 2 polysaccharides, D-trehalose and raffinose; and 6 sugar alcohols, D mannitol, amygdalin, arbutin, esculin ferric citrate, salicin, and gentibulose.

Table 2: Carbohydrate fermentation profile of BNA1 and MED1 using the API 50CHL system

Carbohydrate	BNA1	MED1
0 Control	-	-
1 Glycerol****	+	-
2 Erythritol****	-	-
3 D-arabinose*	+	-
4 L-arabinose*	+	+
5 Ribose*	+	+
6 D-xylose*	-	-
7 L-xylose*	-	-
8 Adonitol****	W	-
9 ß-Methyl- D-Xyloside*	W	-
10 Galactose	+	+
11 D-Glucose*	+	+
12 D-Fructose*	+	+
13 D-Mannose*	+	+
14 L-Sorbose*	-	-
15 Rhaminose*	+	-
16 Dulcitol****	W	-
17 Inositol****	W	-
18 D-Mannitol****	+	+
19 D- Sorbitol****	+	-
20 α-Methyl- D- mannopyranoside*	+	+
21 α-Methyl- D-gluopyranoside*	-	-
22 N-acetyl-glucosamine*	+	+
23 Amygdalin****	+	+
24 Arbutin****	+	+
25 Esculin ferric citrate****	+	+
26 Salicin****	+	+
27 D-Cellobiose**	+	+
28 D-Maltose**	+	+
29 D-Lactose**	+	+
30 D-Melibiose**	W	+
31 Sucrose**	+	+
32 D-Trehalose***	+	+
33 Inulin***	-	-
34 Melezitose***	+	-
35 Raffinose***	-	+
36 Starch***	W	-
37 Glycogen***	W	-
38 Xylitol****	-	-
39 Gentibulose****	+	+
40 D-Turanose***	+	-
41 D-lyxose*	W	-
42 D-tagatose*	W	-
42 D-fucose*	W	-
44 L-fucose*	W	-
45 D-arabitol*	W	-
46 L-arabitol*	W	-
47 Gluconate*	+	-
48 2-Keto-Gluconate****	-	-
49 5-Keto-Gluconate****	+	-

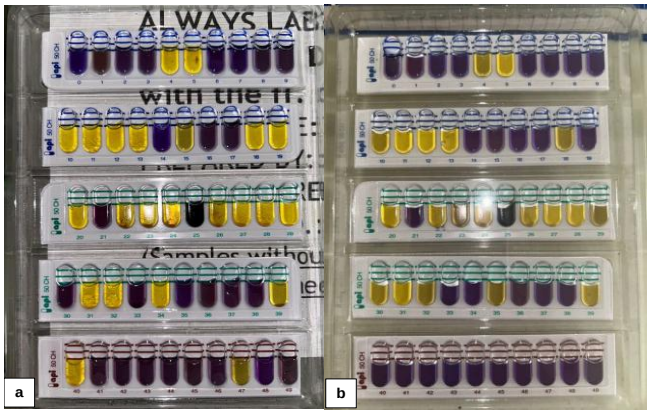

Figure 2: Biochemical Characterization based on API 50 CH System of a) BNA1 and b) MED1 after 48 h of incubation.

The API 50 CH system is a commercially available tool used in clinical and research laboratories to identify LAB and other microorganisms based on their biochemical characteristics.

LAB display diverse carbohydrate fermentation capabilities, commonly fermenting hexoses (e.g., glucose, galactose, mannose, and fructose) and disaccharides (e.g., sucrose, lactose, maltose), occasionally fermenting pentoses (e.g., arabinose, xylose, ribose), yet most LAB generally lacks the enzymes needed to ferment complex polysaccharides such as starch.[33] LAB in plants gains a competitive advantage by swiftly utilizing abundant carbohydrates to produce lactic and acetic acids, thereby suppressing competing microbes.[34] Their evolutionary trajectory, characterized by reduced genome size, crucially enables niche adaptation, albeit at the expense of metabolic efficiency, particularly in electron transfer processes, facilitating their adaptation to iron-limited ecological niches associated with plants or animals.[35] Microorganisms inhabit various parts of plants, including leaves, stems, flowers, fruits, and the root-soil interface, with the numbers of microorganisms ranging from 10^3 to 10^8 cells g^{-1} on the aerial portions and up to 10^{11} cells g^{-1} in the rhizosphere.[36] Flowers, fruits and raw vegetables have not been extensively studied as sources of LAB due to their high carbohydrate content and slightly acidic pH.[37] However, the microbial composition varies depending on intrinsic and extrinsic conditions of the plant matrix.[38-39] Plant-associated LAB species can break down sugars derived from complex plant polysaccharides, tolerate high levels of antimicrobial plant phenolic compounds and use hydroxycinnamic acids as external electron acceptors, with some species possessing a rudimentary electron transport chain that allows them to perform aerobic respiration.[7,36]

Molecular identification of the LAB isolates using 16S rRNA gene

The consensus sequences of BNA1 have a total length of 878 bp (GenBank Acc. No. PP627039), while MED1(GenBank Acc. No. PP627037) is 1,524 bp. Table 3 summarizes the top BLASTN hits for both isolates. BNA1 showed 100% homology to *Lactiplantibacillus plantarum* and 99.89% homology to *Lactiplantibacillus pentosus*,while MED1 showed 99.47% homology with *Enterococcus feacium.* BNA1 was found to cluster with *Lactiplantibacillus pentosus* with 87% bootstrap support (Figure 3), while MED1 grouped with *Enterococcus faecium* with a bootstrap support of 92% (Figure 4).

BLASTN analysis of the 16S rRNA gene successfully identified BNA1 as *Lactiplantibacillus.* However, BNA1 has only one (1) base pair difference with the sequence of *Lactiplantibacillus pentosus* or *Lactiplantibacillus plantarum.* There is difficulty in differentiating these two species using 16s rRNA[40] due to the high sequence identity of these two *Lactiplantibacillus* species[41] and in phenotype characterization[42]. Phylogenetic analysis reveals that BNA1 is more closely linked to *Lpb. Pentosus*, but this could not be verified in this study. The bacterial genus *Lactiplantibacillus* has been observed to exhibit significant plasticity, as evidenced by its high levels of phenotypic and genetic variety, allowing it to adapt effectively.[40] For example, the genes responsible for sugar metabolism exhibit significant variability in response to environmental factors.

Therefore, it is crucial to identify conserved gene areas that remain consistent regardless of environmental fluctuation. It is advisable to conduct additional analysis utilizing another housekeeping gene, such as the *mutL*, to be a superior molecular marker to the previously described[41] housekeeping genes for distinguishing *Lpb. plantarum* species cluster. The use of housekeeping genes like *recA*, *pheS*, *dnaK*, and *rpoA*genes,[40] which can help delineate the two species, is also recommended.

Despite the difficulty in identifying BNA1, it has a promising potential as a LAB. There are over 60 genera of LAB identified, and these include the most common genera used for food fermentation: *Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Enterococcus, Weisella,* and *Lactobacillus* which was recently reclassified into 25 genera.[43] However, it is essential to note that recent revisions in the classification have redefined several *Lactobacillus* species, including *L. pentosus*, now grouped under the genus *Lactiplanctibacillus.*[36]

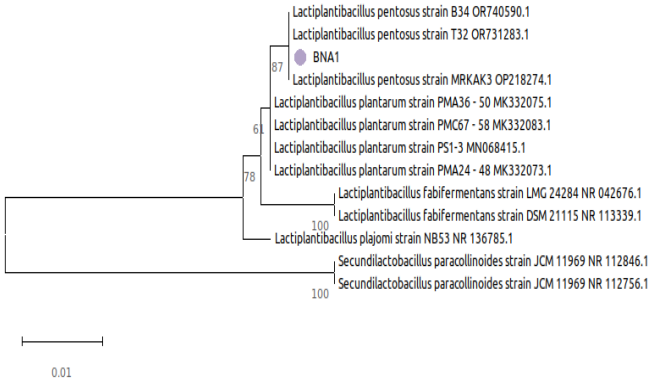

Figure 3: Phylogenetic tree of isolate BNA1 and strains of related species based on 16S rRNA gene sequences. The tree was reconstructed using the maximum-likelihood method. The maximum-likelihood tree is inferred using the K2 substitution model. Node labels indicate bootstrap support values based on 1000 bootstrap replicates.

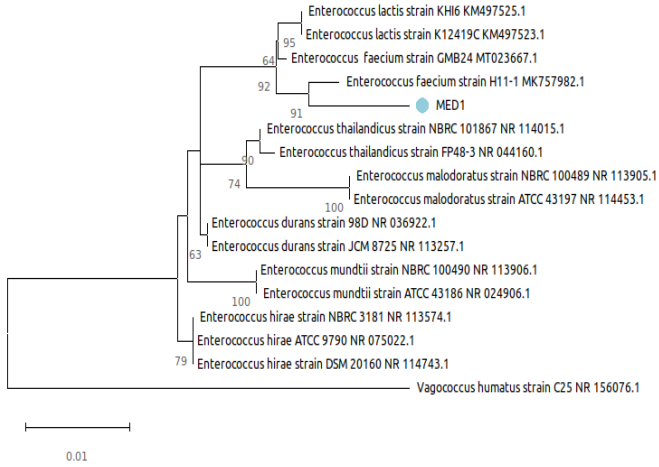

Figure 4: Phylogenetic tree of isolate MED1 and strains of related species based on 16S rRNA gene sequences. The tree was reconstructed using the maximum-likelihood method. The maximum-likelihood tree is inferred using K2+G substitution model. Node labels indicate bootstrap support values based on 1000 bootstrap replicates.

Table 3: Phylogenetic neighbors of LAB isolates on the basis of similarity to the 16S rRNA sequence against BLASTN database.

Scientific Name	Accession Number	Max Score	Total Score	Query Cover	E value	Percent Identity
BNA1						
Lactiplantibacillus plantarum	MK332083.1	1581	1581	99%	0.0	100%
Lactiplantibacillus plantarum	MK332073.1	1581	1581	99%	0.0	100%
Lactiplantibacilluspentosus	MH762173.1	1578	1578	100%	0.0	99.89%
Lactiplantibacillus plantarum	MK333781.1	1578	1578	100%	0.0	99.89%
Lactiplantibacillus plantarum	MK332099.1	1578	1578	100%	0.0	99.89%
MED1						
Enterococcus faecium	MK757982.1	2688	2688	99%	0.0	99.47%
Enterococcus faecium	MT023667.1	2682	2682	99%	0.0	99.41%
Enterococcus faecium	KM095647.1	2667	2667	98%	0.0	99.40%
Enterococcus faecium	KY930926.1	2664	2664	99%	0.0	99.21%
Enterococcus faecium	MK418583.1	2661	2661	98%	0.0	99.33%

*Lactiplantibacillus*species, characterized as homofermentative lactic acid bacteria (LAB), demonstrate efficient fermentation of diverse carbohydrates, including phenolic acids, facilitated by enzymatic processes such as esterase, decarboxylase, and reductase; moreover, these species are distributed across fermented foods, dairy items, insect-related habitats such as plants, and occasionally inhabit the gut microbiota of vertebrates, exhibiting a nomadic ecological pattern.[44] Among the abundant *Lactiplantibacillus* species, *Lpb. plantarum* stands out as a highly versatile strain with valuable characteristics, commonly present in various fermented food items and is extensively utilized in industrial fermentation and the processing of raw food and has been classified as "generally recognized as safe" (GRAS) and holds the status of qualified presumption of safety (QPS).[45] However, attention has increasingly focused on other members of the *Lactiplantibacillus* genus, notably *Lpb. pentosus*, initially described in 1921 and formerly categorized as *Lactobacillus pentosus (Lb. pentosus),*[46] exhibits notable proficiency in efficiently utilizing pentose sugars such as xylose and arabinose for lactic acid production. *Lpb. pentosus* was distinguished from *Lpb. plantarum* strains are based on distinct genotypic features, characterized by rod-shaped cells with rounded ends, typically occurring singly, in pairs, or short chains, consistent with LAB traits.[47] Additionally, *Lpb. pentosus*can hydrolyze various carbohydrates,[47] including amygdalin, l-arabinose, arbutin, cellobiose, d-fructose, galactose, p-gentiobiose, gluconate, d-glucose, glycerol, N-acetylglucosamine, lactose, d-mannose, mannitol, maltose, melibiose, raffinose, ribose, salicin, sorbitol, sucrose, trehalose, and d-xylose, alongside the hydrolysis of esculin, aligning with the API 50 CH findings of test isolate BNA1 in the present investigation. The congruence between the morphological and biochemical characteristics of BNA1 and *Lpb. pentosus* indicates the classification of BNA1 within the *Lpb. pentosus*strains. Several studies have highlighted the probiotic potential of *Lpb. pentosus*, emphasizing its resilience in acidic environments, resistance to bile salts and antibiotics, potent antimicrobial activity against pathogens, non-hemolytic behavior, ability to auto-aggregate and co-aggregate against pathogens, strong adhesion to intestinal cells, biofilm formation, production of exopolysaccharides[48-51] aligning with the criteria set by EFSA and FAO/WHO for identifying lactic acid bacteria as viable probiotic candidates. In alignment with the outcomes of in vitro assessments, various researchers have conducted genomic analyses on select strains of *Lpb. pentosus*, revealing the presence of genes encoding bile salt hydrolases, adhesins, moonlighting proteins, exopolysaccharide (EPS) synthesis machinery, absence of transferable antibiotic resistance genes, and the presence of biosynthetic pathways for seven amino acids, alongside the capacity to ferment a broad array of carbohydrates.[52-54]

On the other hand, MED1 was definitively identified as *Enterococcus faecium* using 16S rRNA gene. *Enterococcus* is a genus of lactic acid bacteria that includes 50 species which are Gram-positive, facultatively anaerobic cocci or short rods which are found in a wide range of environmental ecosystems, including the gastrointestinal tracts of humans and animals, soil, water, and plant environments.[55] The *Enterococcus* genera are known for their capacity to convert carbohydrates into lactic acid through fermentation and have been extensively studied for their potential as probiotics and their role in food fermentation processes; however, certain species within this genus may exhibit pathogenic traits require careful consideration in both clinical and industrial contexts.[56] *E. faecium* SF68 and *E. faecalis* Symbioflor1 are widely recognized and extensively studied as probiotics within the enterococcus genera.[57] The strain *Enterococcus faecalis* Symbioflor 1 clone DSM 16431 was obtained from the intestinal tract of a healthy adult in the 1950s and specifically employed to address various conditions such as urinary tract infections, sinusitis, bronchitis, and irritable bowel syndrome.[55,58] Studies have revealed the capacity of *E. faecalis* as a probiotic, encompassing the generation of biomass and metabolites for the development of flavor and aroma, effective antimicrobial activity against specific pathogens, resilience to acid and bile salt, strong adhesion properties to epithelial cells, and anticancer properties.[57] In a previous study,[59] the researchers used API 50 CH to identify the *E. faecalis* LD33. They found that the isolate was able to ferment 21 different carbohydrates, including glycerol, ribose, galactose, D-glucose, D-Fructose, D-Mannose, D-Mannitol, D-Sorbitol, α-Methyl-D-glucopyranose, N-acetyl-glucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-Cellobiose, D-Maltose, D-Lactose, sucrose, D-Trehalose, and Melezitose. Interestingly, 16 of the 21 carbohydrates fermented by the *E. faecalis* LD33 strain matched the results obtained from the API 50 CH test conducted on MED1 (Table 2). Moreover, the majority of studies examining the probiotic potential of *E. faecalis* have focused on its virulence factors, antibiotic resistance genes, ability to cause disease, and a lack of recognition as Generally Recognized as Safe (GRAS) nor recommended by the Qualified Presumption Safety (QPS) which hinder its use in food, animal, and human health applications.[56,60,61]

Furthermore, the phylogenetic analysis of the 16s rRNA sequences of the isolates showed low (87% – 92%) in bootstrap support, but their grouping with the genus *Lactiplantibacillus* for BNA1 and genus *Enterococcus* for MED1 is seen. BNA1 is grouped with *Lpb.pentosus*, but this does not confirm its species identity because of the low bootstrap support, which confirms previous reports on the difficulty of delineating species of this genus. As a general rule, if the bootstrap

value for a given interior branch is 95% or higher, then the topology at that branch is considered "correct".[62]

Conclusion

The isolates in this study are determined to belong to the LAB group, explicitly belonging to *Lactiplantibacillus* and *Enterococcus* genera, respectively. However, the investigation using 16S rRNA has shown limited ability to discriminate between species within the *Lactiplantibacillus* genus. Notwithstanding this issue, the isolates demonstrated the ability to undergo fermentation of a diverse array of carbohydrates, encompassing monosaccharides, disaccharides, polysaccharides, and sugar alcohols. These characteristics warrant future exploration into the isolates' potential for prebiotic utilization, probiotic properties, and safety assessment.
Additionally, it is strongly advised to employ additional sequencing methods. These include Housekeeping Gene Sequencing, Multilocus Sequence Analysis (MLSA), and Whole Genome Sequencing (WGS). These methods are necessary to accurately and precisely detect and identify the LAB isolates. Furthermore, these allow for analyzing of the genes associated with their probiotic potential and safety. Such genes include virulence genes and antibiotic resistance genes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to acknowledge the Microbiology Laboratory of the Tuklas Lunas Development Center at the Natural Science Research Center at Central Mindanao University for hosting the study. In addition, the authors express their gratitude to the Department of Science and Technology for providing financial support through the Graduate Program - Science and Technology Regional Alliance of Universities for National Development (STRAND) Scholarship, which explicitly aids graduate students. The authors express their gratitude to the assigned reviewers for their commitment and valuable criticism, which significantly improved the quality and presentation of this manuscript, thereby facilitating its publication.

References

1. Leska A, Nowak A, Motyl I. Isolation and Some Basic Characteristics of Lactic Acid Bacteria from Honeybee (*Apis mellifera* L.) Environment—A Preliminary Study. Agriculture. 2022; 12(10):1562. Doi: https://doi.org/10.3390/agriculture12101562
2. Li T, Teng D, Mao R, Hao Y, Wang X, Wang J. A critical review of antibiotic resistance in probiotic bacteria. Food Res. Intl. 2020; 1;136:109571. Doi: https://doi.org/10.1016/j.foodres.2020.109571
3. Food & Agriculture Org. Probiotics in food: Health and Nutritional Properties and Guidelines for Evaluation. Food & Agri Org; 2006.
4. Teneva-Angelova T, Бешкова Д. Non-traditional sources for isolation of lactic acid bacteria. Annals of Micro. 2015; 66(1):449–59. Doi: https://doi.org/10.1007/s13213-015-1127-9
5. Sakandar HA, Kubow S, Sadiq FA. Isolation and in-vitro probiotic characterization of fructophilic lactic acid bacteria from Chinese fruits and flowers. Lebensmittel-Wissenschaft + Technologie/Food Sci & Tech. 2019; 104:70–5. Doi: https://doi.org/10.1016/j.lwt.2019.01.038
6. Maggini V, Mengoni A, Bogani P, Firenzuoli F, Fani R. Promoting model systems of Microbiota–Medicinal plant interactions. Trends Plant Sci. 2020; 25(3):223–5. Doi: https://doi.org/10.1016/j.tplants.2019.12.013
7. Filannino P, Di Cagno R, Gobbetti M. Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth. Curr. Opin. Biotechnol; 49:64–72. Doi: https://doi.org/10.1016/j.copbio.2017.07.016
8. Rodríguez LGR, Mohamed F, Bleckwedel J, Medina RB, De Vuyst L, Hébert EM, et al. Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in northern Argentina. Front. Microbiol. 2019; 10. Doi: https://doi.org/10.3389/fmicb.2019.01091
9. Saguibo JD, Mercado MA, Maldia ST, Jimeno BT, Perez MTM, Calapardo MR, et al. Identification and characterization of lactic acid bacteria isolated from some medicinal and/or edible Philippine plants. Food Res. 2019; 698–712. Doi: https://doi.org/10.26656/fr.2017.3(6).148
10. Divisekera DMWD, Samarasekera JKRR, Hettiarachchi C, Gooneratne J, Choudhary M, Gopalakrishnan S. Isolation and identification of lactic acid bacteria with probiotic potential from fermented flour of selected banana varieties grown in Sri Lanka. Natl. Sci. Found. 2019; 47(1):3. Doi: https://doi.org/10.4038/jnsfsr.v47i1.8922
11. Bagabaldo PA, Atienza L, Castillo-Israel KAT, Barrion ASA, Laurena AC, Estacio MAC. Exploring the Nutritional, Antioxidant, and Lipid-Lowering Properties of Saba Banana (*Musa acuminata x balbisiana* BBB Group) Peels. Phil. Agric. Sci. 2023; 106(4):358–68. Doi: https://doi.org/10.62550/ci037023
12. Chowdary MY, Rana SS, Ghosh P. Banana inflorescence and their potential health benefits as future food. Qual. Assur. Saf. Crops Foods. 2022; 14(2):131–6. Doi: https://doi.org/10.15586/qas.v14i2.1066
13. Zou F, Tan C, Zhang B, Wu W, Shang N. The Valorization of Banana By-Products: Nutritional composition, bioactivities, applications, and future development. Foods. 2022; 11(20):3170. Doi: https://doi.org/10.3390/foods11203170
14. Rayos AL, Rodriguez EB, Gruezo WS, Hadsall AS, Cardenas LB. Phenetic analysis of eighteen species of Philippine *Medinilla* Gaudich. (Melastomataceae) based on morphological characteristics and phenolic profile. Phil. J. Syst. Biol. 2016; 10:14-24.
15. Han QH, Ye M, Gong X, Zhang N, Zhang C, Li M. Chemical constituents of *Medinilla septentrionalis* (W. W. Sm.) H. L. Li (Melastomataceae). Biochem. Syst. Ecol. 2019; 86:103901. Doi: https://doi.org/10.1016/j.bse.2019.05.009
16. Milanda T, Fitri WN, Barliana MI, Chairunnisaa AY, Sugiarti L. Antifungal activities of *Medinilla speciosa* Blume fruit extracts against Candida albicans and Trichophyton rubrum.J. Adv. Pharm. Educ. 2021; 11(3):1–8. Doi: https://doi.org/10.51847/xdbihmqd2p
17. De Man JC, Rogosa M, Sharpe ME. A Medium For The Cultivation Of Lactobacilli. J. Appl. Bacteriol. 1960; 23(1):130–5. Doi: https://doi.org/10.1111/j.1365-2672.1960.tb00188.x
18. Yudianti NF, Yanti R, Cahyanto MN, Rahayu ES, Utami T. Isolation and Characterization of Lactic Acid Bacteria from Legume Soaking Water of Tempeh Productions. Digi. Press Life Sci. 2020; 2:00003. Doi: https://doi.org/10.29037/digitalpress.22328
19. Omar HJG, Regio MGM, Peña JCD, Lim NT, Andal MS, De Guzman GQ, Santiago NCD, Ecalne NJKT, De Guzman NLMC, Montemayor NSS, Lapig NJDR, Ramos NJPDT. Proximate composition and antioxidant activity of the saba (*Musa acuminata x balbisiana* Colla) banana blossom family Musaceae. GSC Biol. Pharm. Sci. 2022; 21(2):026–32. Doi: https://doi.org/10.30574/gscbps.2022.21.2.0418

20. Ramu R, Shirahatti PS, Anilakumar KR, Nayakavadi S, Zameer F, Dhananjaya BL, Prasad, MNN. Assessment of nutritional quality and global antioxidant response of banana (*Musa* sp. CV. Nanjangud Rasa Bale) pseudostem and flower. Pharmacogn. Res. 2017; 9(5):74. Doi: https://doi.org/10.4103/pr.pr_67_17
21. Soni D, Saxena G. Complete nutrient profile of Banana flower: A review.J. Plant Sci. Res. 2021; 37(2):263–7. Doi: https://doi.org/10.32381/jpsr.2021.37.02.6
22. Kavya MN, Manasa R, Deepika M, Shivananjappa M, Shekhara Naik R. A review on banana flower: Nutritional composition, processed products and health benefits. IP J. Nutr. Metab. Health Sci. 2023; 6(3):110–5. Doi: https://doi.org/10.18231/j.ijnmhs.2023.019
23. Guan Z, Yu EZ, Feng Q. Soluble dietary fiber, one of the most important nutrients for the gut microbiota. Molecules 2021; 26(22):6802. Doi: https://doi.org/10.3390/molecules26226802
24. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, Berenjian A, Ghasemi Y. Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods. 2019;8(3):92. Doi: https://doi.org/10.3390/foods8030092
25. Ulloa CU, Almeda F, Goldenberg R, Kadereit G, Michelangeli FA, Penneys DS, Stone RD, Veranso-Libalah MC. Melastomataceae: Global Diversity, distribution, and endemism. Syst. Evol. Ecol. Melastomataceae, Springer, Cham. 2022; 3–28. Doi: https://doi.org/10.1007/978-3-030-99742-7_1
26. Fernando ES, and Balete DS. *Medinilla dallciana* (Melastomataceae: Dissochaeteae), a new species from Luzon Island, Philippines. Phil. J. Sci. 2013;142(Special Issue), 88–93.
27. Quakenbush PJ. Pollination, Mating System, Phenology and Characterisation of *Medinilla multiflora*Merr. (Melastomataceae) on Mt Makiling, Philippines. Int. J. Bot. Gard. Hort. 2018; 16: 121–139. Doi: Doi: https://doi.org/10.24823/Sibbaldia.2018.251
28. Han QH, Ye M, Gong X, Zhang N, Zhang C, Li MH. Chemical constituents of *Medinilla septentrionalis* (W. W. Sm.) H. L. Li (Melastomataceae). Biochem. Syst. Ecol. 2019; 86:103901. Doi: https://doi.org/10.1016/j.bse.2019.05.009
29. Milanda T, Fitri WN, Barliana MI, Chairunnisaa AY, Sugiarti L. Antifungal activities of *Medinilla speciosa* Blume fruit extracts against *Candida albicans* and *Trichophyton rubrum.* J. Adv. Pharm. Educ. Res. 2021; 11(3):1–8. Doi: https://doi.org/10.51847/xdbihmqd2p
30. Banaay CGB, Balolong MP, Elegado FB. Lactic acid bacteria in Philippine traditional fermented foods. IntechOpen. 2013. Doi: https://doi.org/10.5772/50582
31. Martínez B, RodríGuez A, Kulakauskas S, Chapot- Chartier M. Cell wall homeostasis in lactic acid bacteria: threats and defences. FEMS Microbiol. Rev. 2020; 44(5):538–64. Doi: https://doi.org/10.1093/femsre/fuaa021
32. Fidan H, Esatbeyoglu T, Šimat V, Trif M, Tabanelli G, Kostka T, Montanari C, Ibrahim SA, Özogul F. Recent developments of lactic acid bacteria and their metabolites on foodborne pathogens and spoilage bacteria: Facts and gaps. Food Biosci. 2022; 47:101741. Doi: https://doi.org/10.1016/j.fbio.2022.101741
33. Gunkova PI, Buchilina AS, Максимюк НН, BazarnovaYuG, Girel KS. Carbohydrate fermentation test of lactic acid starter cultures. OP Conf. Ser. Earth Environ. Sci. 2021; 852(1):012035. Doi: https://doi.org/10.1088/1755-1315/852/1/012035
34. Cui Y, Qu X. Genetic mechanisms of prebiotic carbohydrate metabolism in lactic acid bacteria: Emphasis on *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei* as flexible, diverse and outstanding prebiotic carbohydrate starters. Trends Food Sci. Technol. 2021; 115:486–99. Doi: https://doi.org/10.1016/j.tifs.2021.06.058
35. Gänzle MG. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr. Sci. 2023; 6(3):110–5. Doi: https://doi.org/10.18231/j.ijnmhs.2023.019
36. Yu A, Leveau JHJ, Marco ML. Abundance, diversity and plant- specific adaptations of plant- associated lactic acid bacteria. Environ. Microbiol. Rep. 2019 Oct 28; 12(1):16–29. Doi: https://doi.org/10.1111/1758-2229.12794
37. Endo A, Tanizawa Y, Arita M. Isolation and Identification of Lactic Acid Bacteria from Environmental Samples. Meth. Mol. Biol 2018; 1887:3–13. Doi: https://doi.org/10.1007/978-1-4939-8907-2_1
38. Di Cagno R, Coda R, De Angelis M, Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiol. 2013; 33(1):1–10. Doi: https://doi.org/10.1016/j.fm.2012.09.003
39. Garcia EF, Luciano WA, Xavier DE, Da Costa WKA, De Sousa Oliveira K, Franco OL, De Morais Júnior MA, Lucena, BTL, Picão RC, Magnani M, Saarela M, De Souza, EL. Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected *Lactobacillus* strains. Front. Microbiol. 2016; 7. Doi: https://doi.org/10.3389/fmicb.2016.01371
40. Skotniczny M, Satora P. Molecular Detection and Identification of Plant-Associated *Lactiplantibacillus plantarum*. Int. J. Mol. Sci. 2023; 24(5):4853. Doi: https://doi.org/10.3390/ijms24054853
41. Huang C, Chen CC, Lin Y, Chen CH, Lee AY, Liou J, Gu C, Huang L.The *mutL* Gene as a Genome-Wide Taxonomic Marker for High Resolution Discrimination of *Lactiplantibacillus plantarum* and Its Closely Related Taxa. Microorganisms. 2021; 9(8):1570. Doi: https://doi.org/10.3390/microorganisms9081570
42. Torriani S, Felis GE, Dellaglio F. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA*Gene Sequence Analysis and Multiplex PCR Assay with *recA* Gene-Derived Primers. Appl. Environ. Microbiol. 2001; 67(8):3450–4. Doi: https://doi.org/10.1128/aem.67.8.3450-3454.2001
43. Wang Y, Wu Y, Wang Y, Han X, Mei X, Yu D, Wang Y, Li W. Antioxidant properties of probiotic bacteria. Nutrients. 2017; 9(5):521. Doi: https://doi.org/10.3390/nu9050521
44. Zheng J, Wittouck S, Salvetti E, Franz CM a. P, Harris HMB, Mattarelli P, O' Toole PW, Pot B, Vandamme, P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol. 2020; 70(4):2782–858. Doi: https://doi.org/10.1099/ijsem.0.004107
45. Behera SS, Ray RC, Zdolec N. *Lactobacillus plantarum* with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. Biomed Res. Int. 2018; 2018:1–18. Doi: https://doi.org/10.1155/2018/9361614
46. Fred EB, Peterson WH, Anderson JA. The characteristics of certain pentose-destroying bacteria, especially as concerns their action on arabinose and xylose. J. Biol. Chem. 1921; 48(2):385–412. Doi: https://doi.org/10.1016/s0021-9258(18)86020-3
47. Zanoni P, Farrow J a. E, Phillips BA, Collins MD. *Lactobacillus pentosus* (Fred, Peterson, and Anderson) sp. nov., nom. rev. Int. J. Syst. Bacteriol. 1987; 37(4):339–41. Doi: https://doi.org/10.1099/00207713-37-4-339
48. Abriouel H, Benomar N, Lucas R, Gálvez A. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally-fermented Aloreña green table olives. Int. J. Food Microbiol. 2011;

144(3):487–96. Doi: https://doi.org/10.1016/j.ijfoodmicro.2010.11.006
49. Pavli F, Argyri AA, Papadopoulou OS. Probiotic Potential of Lactic Acid Bacteria from Traditional Fermented Dairy and Meat Products: Assessment by In Vitro Tests and Molecular Characterization. J. Probiotics Health. 2016; 04(03). Doi: https://doi.org/10.4172/2329-8901.1000157
50. Al-Yami AM, Al-Mousa AT, Al-Otaibi SA, Khalifa A. *Lactobacillus* species as probiotics: isolation sources and health benefits. J. Pure Appl. Microbiol. 2022; 16(4):2270–91. Doi: https://doi.org/10.22207/jpam.16.4.19
51. Gu M, Cho JH, Suh J, Cheng J. Potential oral probiotic *Lactobacillus pentosus* MJM60383 inhibits *Streptococcus mutans* biofilm formation by inhibiting sucrose decomposition. J. Oral Microbiol. 2022; 15(1). Doi: https://doi.org/10.1080/20002297.2022.2161179
52. Abriouel H, Montoro BP, Del Carmen Casado Muñoz M, Lerma LL, Pestaña MH, Gómez NC, Franz CMaP, Gálvez A, Benomar N. Complete Genome Sequence of a Potential Probiotic, *Lactobacillus pentosus* MP-10, Isolated from Fermented Aloreña Table Olives. Genome Announc. 2016; 4(5). Doi: https://doi.org/10.1128/genomea.00854-16
53. Abriouel H, Montoro BP, Casimiro- Soriguer CS, Pérez-Pulido AJ, Knapp CW, Gomez NC, Castillo-Gutiérrez S, Estudillo-Martínez MD, Gálvez A, Benomar N. Insight into Potential Probiotic Markers Predicted in *Lactobacillus pentosus* MP-10 Genome Sequence. Front. Microbiol. 2017; 8. Doi: https://doi.org/10.3389/fmicb.2017.00891
54. Kai Y, Li P, Gu Q. Complete genome sequence analysis of a strain *Lactobacillus pentosus* ZFM94 and its probiotic characteristics. Genomics. 2020; 112(5):3142–9. Doi: https://doi.org/10.1016/j.ygeno.2020.05.015
55. Krawczyk B, Wityk P, Gałęcka M, Michalik M. The Many Faces of *Enterococcus* spp.—Commensal, Probiotic and Opportunistic Pathogen. Microorganisms. 2021; 9(9):1900. Doi: https://doi.org/10.3390/microorganisms9091900
56. Hanchi H, Mottawea W, Sebei K, Hammami R. The genus *Enterococcus*: Between Probiotic Potential and Safety Concerns—An update. Front. Microbiol. 2018; 9. Doi: https://doi.org/10.3389/fmicb.2018.01791
57. Jiao Y, Yang HM, Shigwedha N, Zhang S, Liu F, Zhang L. Probiotic Effects and Metabolic Products of *Enterococcus faecalis* LD33 with Respiration Capacity. Foods. 2022; 11(4):606. Doi: https://doi.org/10.3390/foods11040606
58. Baccouri O, Boukerb AM, Farhat LB, Zebré A, Zimmermann K, Domann E, Cambronel M, Barreau M, Maillot O, Rincé I, Muller C, Marzouki MN, Feuilloley M, Abidi F, Connil N. Probiotic Potential and Safety Evaluation of *Enterococcus faecalis* OB14 and OB15, Isolated from Traditional Tunisian Testouri Cheese and Rigouta, Using Physiological and Genomic Analysis. Front. Microbiol. 2019;10. Doi: https://doi.org/10.3389/fmicb.2019.00881
59. Jiao YH, Zhang L, Liu F. Identification of a Lactic Acid Bacteria Strain from Traditional Dairy Products. Adv. Mater. Res. 2013;781–784:1599–602. Doi: https://doi.org/10.4028/www.scientific.net/amr.781-784.1599
60. Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Klein G, Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gómez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C, Fernández Escámez PS. Scientific Opinion on the update of the list of QPS- recommended biological agents intentionally added to food or feed as notified to EFSA†. EFSA Journal. 2017; 15(3). Doi: https://doi.org/10.2903/j.efsa.2017.4664
61. Sonei A, Dovom MRE, Yavarmanesh M. Evaluation of probiotic, safety, and technological properties of bacteriocinogenic *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from lighvan and koozeh cheeses. Int. Dairy J. 2024; 148:105807. Doi: https://doi.org/10.1016/j.idairyj.2023.105807
62. Kumar S, Filipski A, Battistuzzi FU, Pond SLK, Tamura K. Statistics and truth in phylogenomics. Mol. Biol. Evol. 2011; 29(2):457–72. Doi: https://doi.org/10.1093/molbev/msr202