World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(Research Article)

Isolation and molecular characterization of lactic acid bacteria isolated from selected fermented food condiments

Ogunleke O. B * and Oladipo I. C

Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

World Journal of Biology Pharmacy and Health Sciences, 2025, 23(01), 064-070

Publication history: Received on 27 May 2025; revised on 01 July 2025; accepted on 04 July 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.23.1.0634

Abstract

This study investigated the isolation and molecular characterization of lactic acid bacteria (LAB) from four samples of fermented food condiments in Nigerian; Samples are fermented unsoften locust beans (*iru woro*), fermented soften locust beans (*Iru pete*), fermented African oil bean seeds (*Ugba*), fermented melon seed (*Cirulus colocynthis*) (*Ogiri*). Samples were obtained from Sabo market in Ogbomosho, Oyo State, and processed according to standard microbiological techniques on MRS medium under anaerobic conditions. Total LAB counts ranged from 1.2×10^6 to 1.9×10^6 CFU/g, with the highest count in the fermented unsoften locust beans (*iru woro*). Five distinct LAB isolates were obtained and molecularly characterized via 16S rRNA gene sequencing. From the BLAST analysis, with a similarity of 99-100% to the reference strains, the isolates were identified as *Lactobacillus acidophilus* IR1, *Lactobacillus delbrueckii* UG1, *Lactobacillus johnsonii* OG1, *Lactococcus garvieae* OG2, and and *Lactococcus lactis* IW1. All the isolates have been deposited in NCBI GenBank under accession numbers PQ758599, PQ758600, PQ555637, PQ555638, and PQ555639, respectively. The phylogenetic analysis showed that the isolates clustered within the *Lactobacillaceae* family and had a close relationship to other known probiotic strains. This shows the very high diversity that exists among the beneficial LAB in traditional Nigerian fermented foods and their availability for further studies in food biotechnology and probiotic development.

Keywords: Fermented Food Condiments; *Lactobacillus*; Lactic Acid Bacteria; Probiotics

1. Introduction

Lactic acid bacteria (LAB) have been a crucial component in the fermentation process for centuries, contributing significantly to the development of flavor and chemical changes in various fermented foods [1]. The term "Lactic Acid Bacteria" was first widely accepted in the early 20th century, replacing earlier terms like "milk souring" and "lactic acid producing" bacteria, which caused confusion among researchers [2, 3]. The work of Orla-Jensen in 1919 and Axelsson in 1989 played a significant role in the systematic classification of LAB [2]. Lactic acid bacteria (LAB) represent a diverse group of Gram-positive, catalase-negative, facultatively anaerobic microorganisms that play crucial roles in food fermentation processes worldwide [4]. These bacteria are characterized by their ability to produce lactic acid as the primary end product of carbohydrate metabolism, making them essential for the preservation, flavor development, and nutritional enhancement of numerous fermented foods [5, 6]. The significance of LAB extends beyond their traditional role in food preservation to encompass probiotic properties, antimicrobial compound production, and potential applications in biotechnology and pharmaceutical industries [7]. The traditional fermented condiments serve not only as flavor enhancers but also as repositories of indigenous microbial diversity, harboring unique strains of LAB that have adapted to specific environmental conditions and substrate compositions over centuries of traditional fermentation practices [8]. The molecular characterization of LAB has revolutionized our understanding of their taxonomy, phylogenetic relationships, and functional diversity. Traditional phenotypic identification methods, while useful, often

---

[*] Corresponding author: Ogunleke O. B

lack the precision required for accurate species-level identification and strain differentiation [9,10]. Modern molecular techniques, including 16S rRNA gene sequencing, multi-locus sequence typing (MLST), and whole-genome sequencing, have provided powerful tools for the precise identification and characterization of LAB isolates. The 16S rRNA gene sequencing approach has become the gold standard for bacterial identification due to its universal presence, functional constancy, and appropriate sequence variation for phylogenetic analysis. Additionally, molecular fingerprinting techniques such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Restriction Fragment Length Polymorphism (RFLP) provide valuable tools for strain-level differentiation and population structure analysis [11].

The study of LAB diversity in fermented food condiments is particularly relevant in the context of increasing consumer demand for natural, functional foods and the growing interest in traditional fermentation practices. Many traditional fermented condiments are produced through spontaneous fermentation processes that rely on indigenous microbial populations, making them valuable sources of novel LAB strains with unique properties. The characterization of these strains not only contributes to scientific knowledge but also supports the preservation of traditional food culture and potentially leads to the development of improved fermentation processes. Despite the significant progress in LAB research, many fermented food condiments remain underexplored in terms of their microbial composition and diversity. The isolation and molecular characterization of LAB from these products represent an important step toward understanding the complex microbial ecology of fermented foods and unlocking their potential for various biotechnological applications [12, 13]. Lactic acid bacteria are a major group of commensal bacteria, with certain strains exhibiting probiotic properties, particularly *Lactobacilli*, which are commonly found in fermented foods [14]. Many LAB strains isolated from fermented foods have been demonstrated to be beneficial to humans and have potential as probiotics [15]. The identification and classification of LAB populations have traditionally relied on phenotypic, biochemical, and physiological tests. However, these methods have limitations, such as the complexity of procedures, varying nutritional and growth needs of LAB, and lower discriminatory power [16]. The emergence of molecular tools has addressed these limitations, enabling the accurate identification of LAB strains using PCR amplification, DNA sequencing, and other genotypic methods [17]. There is an increased interest in probiotics, in face of their recorded safe use and recognized effects on human health [2]. Probiotics are live microorganisms when administered in adequate quantities; improve the health of the host [3].

Lactic acid bacteria (LAB) can be considered among the useful microbes present in fermented food condiments [18]. They are rod- or cocci-shaped, non-sporulating, Gram-positive bacteria with very high pH tolerance, carrying out essentially the fermentation of carbohydrates to yield lactic acid as the principal end product [5]. Proteins and lipids are also biodegraded by these bacteria. Lactic acid bacteria also produce alcohols, esters, aldehydes, and some flavoring compounds such as sulfur compounds during the fermentation of various foods [16]. Lactic acid bacteria comprise over 61 genera, among which *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus*, and *Pediococcus* are major inhabitants of fermented foods [18]. Recently, *Leuconostocaceae* and *Lactobacillaceae* were merged into the family *Lactobacillaceae*, while the genus *Lactobacillus* was divided into 25 genera [19, 18]. Beneficial and edible lactic acid bacteria are classified as probiotics [20], though they do not exclusively represent this category. Nevertheless, LAB constitutes the predominant group within probiotic microorganisms and provides significant health benefits to humans. This study aimed to isolate and molecularly characterize lactic acid bacteria from four traditional Nigerian fermented condiments.

2. Materials and Methods

2.1. Sample Collections and preparation

The fermented food condiments were collected from Sabo Market (8° 8' 31.7940'' N and 4° 14' 42.6696'' E) in Ogbomosho, Oyo State. The samples included fermented unsoften locust beans (*iru woro*), fermented soften locust beans (*Iru pete*), fermented African oil bean seeds (*Ugba*), and fermented melon seeds (*Citrullus colocynthis*) used to produce *Ogiri*. All samples were collected in sterile polythene bags and transported to the laboratory in ice packs to preserve microbial integrity. One gram of each sample was aseptically transferred to 9 mL of sterile peptone water, gently shaken, and allowed to homogenize for 30 minutes. Ten-fold serial dilutions were prepared from the sample homogenate and streaked onto duplicate sterile petri dishes containing MRS (De Man, Rogosa and Sharpe) agar. The inoculated plates were incubated anaerobically at 30 °C for 2-3 days in an anaerobic incubator. After 72 hours of incubation, the plates were examined for visible colonies, which were subsequently sub-cultured to obtain pure cultures. Fresh MRS medium was prepared and sterilized at 121 °C for 15 minutes, allowed to cool, and then aseptically poured into sterilized petri dishes. The bacterial isolates were sub-cultured onto fresh plates using the streaking method to obtain pure, distinct colonies. The plates were incubated at 37 °C for an additional 72 hours and examined for pure and distinct colony formation.

2.2. Molecular Characterization of the Isolates

Fresh cultures of the bacterial isolates were subjected to DNA extraction using the EZ-10 Spin Column Bacterial Genomic DNA Miniprep Kit (Bio Basic, Markham, Canada). The integrity and concentration of the extracted DNA were determined through optical density measurements using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) [21].

2.3. Amplification and Sequencing of 16S rRNA Gene Using PCR

The 16S rRNA genes were amplified using Polymerase Chain Reaction (PCR) using bacterial universal primers (27F–AGAGTTTGATCCTGGCTCAG and 1492R– GGTTACCTTGTTACGACTT). The PCR reaction was conducted in a Techne PTC-100 Thermal Cycler, involving denaturation, 30 cycles of amplification, and extension. The PCR products were separated by electrophoresis on a 1% agarose TAE gel and visualized by UV transillumination. The amplicons were purified, sequenced for the 16S gene, and BLAST searched for similar sequences in the NCBI database. A phylogenetic tree was plotted to understand evolutionary relationships among organisms [17].

3. Results and Discussion

3.1. Isolation and Molecular Identification of the Isolates

The total *Lactobacillus* counts from fermented unsoften locust beans (*iru woro*), fermented soften locust beans (*iru pete*), fermented African oil bean seeds (*ugba*), and fermented melon seeds (*Citrullus colocynthis*) used to produce *ogiri* samples used in the study (Figure 1) are shown in Table 1, it was observed that the highest colony count was observed in fermented unsoften locust beans (*iru woro*) with 1.9 × 10^6 CFU/g, while *ugba* exhibited the lowest colony count of 1.0 × 10^6 CFU/g. In this study, the highest colony count was observed in fermented unsoften locust beans (*iru woro*) with a colony-forming unit of 1.9 × 10^6 CFU/g, while *ugba* had the lowest colony count of 1.0 × 10^6 CFU/g. It was also reported that the highest colony count of 2.0 × 10^4 CFU/g was gotten in *ogiri*; Olanbiwoninu and Odunfa [22] also reported colony counts of 1.5 × 10^6 and 2.0 × 10^6 CFU/g in *iru* and *ogiri-egusi*, thus aligning closely with the current study's range.

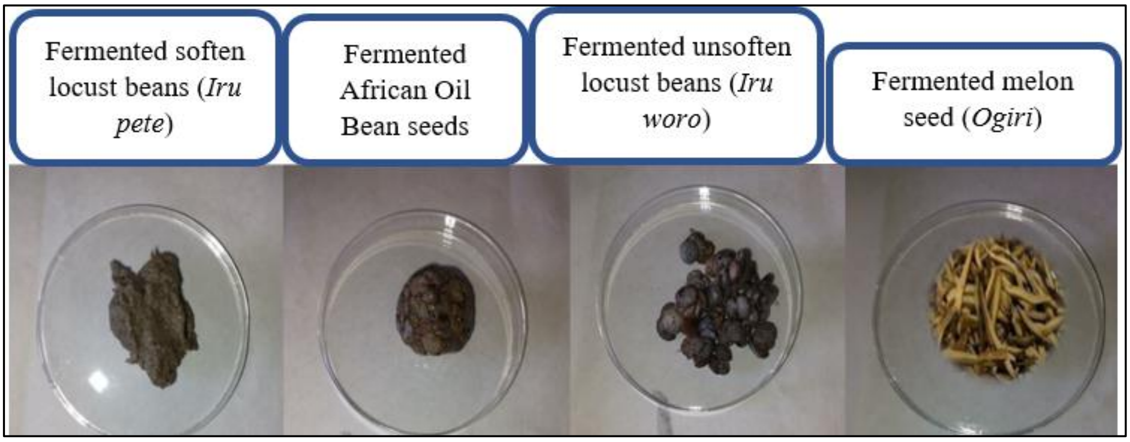


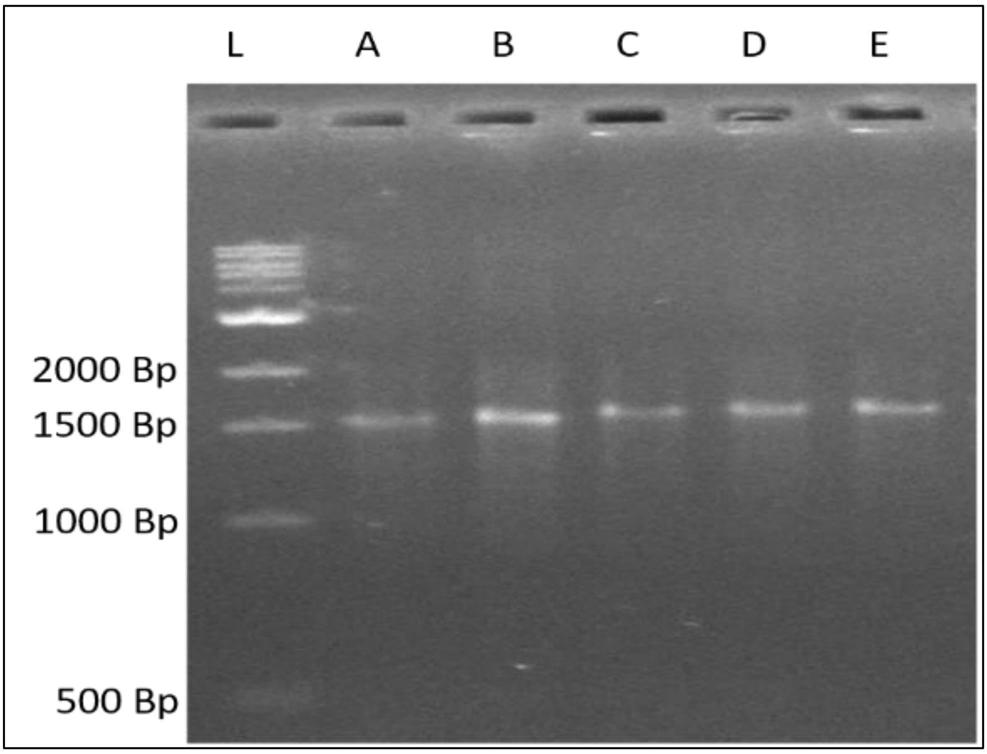
Figure 1 Some of the fermented food condiments used in the study

Table 1 The microbial load of the samples

Samples	Total Lactic Acid Bacteria Count (CFU/g)
IW	1.9 x 10^6
IR	1.6 x 10^6
UG	1.0 x 10^6
OG	1.2 x 10^6

Key: IR: *Iru pete*, UG: Ugba, OG: *Ogiri*, IW: *Iru Woro*. CFU/g: colony forming unit per gram.

After PCR, the amplified gene on 1.5% agarose gel shows distinct single DNA band with molecular weight of 1500bps which correspond to the expected amplicon size is presented in Figure 2. The isolates were identified using targeted unique sequences (1500 bps length) that were aligned using Bio Edit sequence alignment editor version 7.2.5.0. The 16S rRNA gene sequences obtained demonstrated 99%–100% similarity to reference sequences in the NCBI database following BLAST analysis. The isolates were identified as *L. acidophilus* IR1, *L. delbrueckii* UG1, *Lactobacillus johnsonii* OG1, *Lactococcus garvieae* OG2, and *Lactococcus lactis* IW1, were deposited in the NCBI GenBank database and assigned accession numbers PQ758599, PQ758600, PQ555637, PQ555638, and PQ555639, respectively (Table 2). Olanbiwoninu and Odunfa [22] also reported the isolation and molecular characterization of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Pediococcus cellicola* from fermented condiments. Yudha *et al.* [23] also reported the isolation and molecular characterization of *L. brevis* and *L. lactis* from *Budu*. Oladipo *et al.* [5] also emphasized the significant role and abundance of *Lactobacillus* species in fermented foods and condiments.



Lane A – E: IR1, UG1, OG1, OG2, IW1; L = Molecular ladder (500 bp)

Figure 2 Amplified 16s rRNA gene of the LAB isolates

The phylogenetic relationship among the bacterial isolates is illustrated in Figure 3. The isolates predominantly cluster within the *Lactobacillaceae* family, showing close evolutionary relationships with *Lactobacillus acidophilus* (AB690249.1), *Lactobacillus delbrueckii* (AB690241.1), *Lactobacillus johnsonii* (LC071811.1), *Lactococcus garvieae* (LC145570.1), and *Lactococcus lactis* (LC311737.1). The isolates derived from the fermented food condiments samples formed a well-supported monophyletic clade that, while distinct, demonstrates close phylogenetic affinity to established reference strains retrieved from the NCBI database. The phylogenetic analysis revealed high sequence similarities between the isolates and reference strains. Isolate IR1 demonstrated 99% homology with *Lactobacillus acidophilus* NBRC strain, while UG1 showed 99.6% homology with *L. delbrueckii* NBRC 13951 strain (Iqbal *et al.*, [24], OG1 exhibited 99% homology with *L. johnsonii* R094 strain (Mulaw *et al.*, [25]), OG2 displayed 99% homology with *Lactococcus garvieae* YIT 0080 strain and IW1 showed 99% homology with *Lactococcus lactis* DSM 13335 (Mulaw *et al.*, [25]). Previous studies have emphasized the importance of fermented food condiments as valuable sources for the continuous isolation of lactic acid bacteria, attributed to their ability to produce exceptionally high levels of lactic acid and maintain viability under highly acidic conditions [26]. These microorganisms have been documented to exist extensively across diverse habitats, including plants, dairy environments, and a wide range of commercially important fermented products globally, as reported by David *et al.* [26]. Huligere *et al.* [27] noted that *Lactobacillus* species represent one of the most predominant LAB groups with high occurrence rates when isolated from dairy and plant sources through fermentation processes.

Table 2 Molecular Identity and Accession Numbers of the Isolates

Code	Sources	Identity	Accession number
IR1	Fermented soften locust beans (*iru pete*)	*Lactobacillus acidophilus*	PQ758599
UG1	Fermented African oil bean seeds (*ugba*)	*Lactobacillus delbrueckii*	PQ758600
OG 1	Fermented melon seeds (*Citrulus colocynthis*) (*ogiri*) 1	*Lactobacillus johnsonii*	PQ555637
OG 2	Fermented melon seeds (*Citrulus colocynthis*) seeds (*ogiri*) 2	*Lactococcus garvieae*	PQ555638
IW1	fermented unsoften locust beans (*iru woro*),	*Lactococcus lactis*	PQ555639

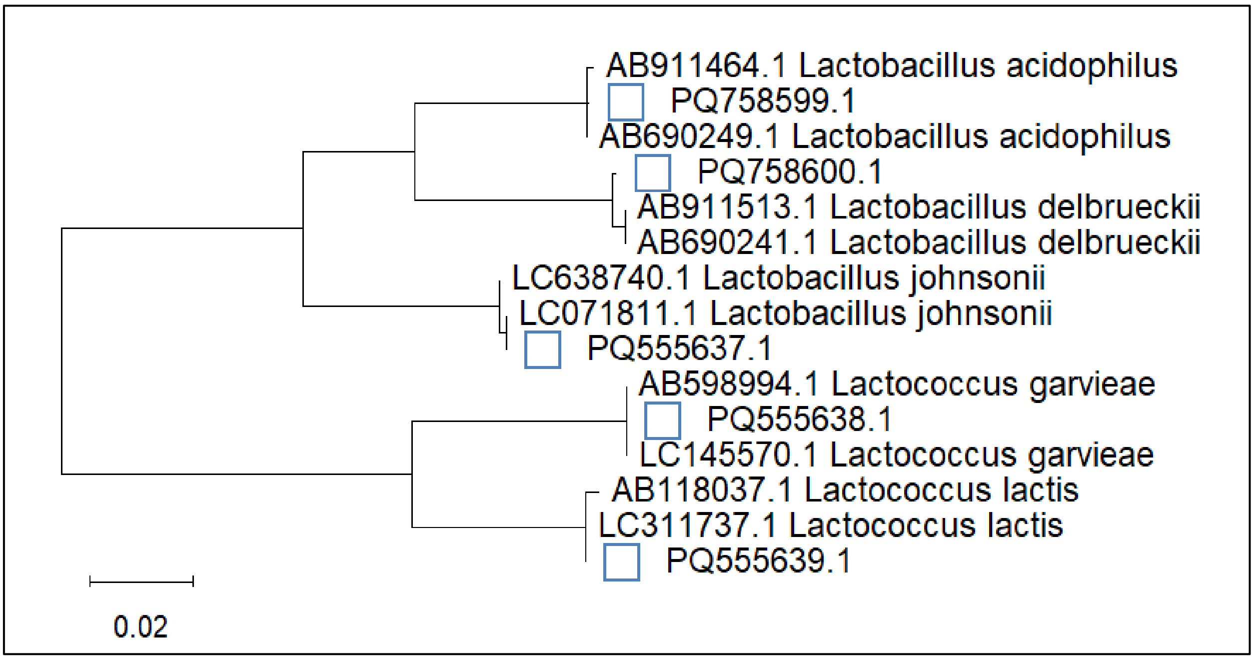


Figure 3 Phylogenetic tree showing the evolutionary relationship between the LAB genes sequence detected (1000 bootstrap) and the gene sequence detected in the GenBank

4. Conclusion

In this study five different LAB strains from Nigerian traditional fermented food condiments were successfully isolated, molecularly characterized and deposited in NCBI GenBank and accession numbers were assigned. The phylogenetic analysis revealed evolutionary connections between the LAB gene sequences and those catalogued in GenBank, demonstrating how this research integrates traditional understanding with modern molecular techniques. This study primarily contributes to SDG 2 (zero hunger) and SDG 3 (good health and well-being). The isolates in the study are crucial for food preservation and safety, enhancing nutritional value, and thus offering probiotic potentials, all of which support food security and public health.

Compliance with ethical standards

Acknowledgements

The authors wish to acknowledge the management of Ladoke Akintola University of Technology Ogbomoso, Oyo State for the provision of the facilities for this research.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Oladipo IC, Oyewumi MM. The Role of Lactic Acid Bacteria in Food Processing, Nutrition and Human Health. 2024; International Journal of Current Microbiology and Applied Sciences 13 (10): 288 – 296. https://doi.org/10.20546/ijcmas.2024.1310.033

[2] Khalid K. An overview of lactic acid bacteria. International Journal of Biosciences, 2011. 1(3): 1–13.

[3] Coelho MC, Malcata FX, Silva CC. Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. Foods. 2022; 11(15):22-76.

[4] Holzapfel HW, Haberer P, Geisen R, Bjorkroth J, Schillinger U. Taxonomy and important features of probiotic microorganisms in food and nutrition. 2001; DOI: 10.1093/ajcn/73.2.365s, PMID: 11157343.

[5] Oladipo IC. Safety of lactic acid bacteria in foods: A perspective. 2025a; Food and Health 7(1):1. https://doi.org/10.53388/FH2025001.

[6] Axelsson L. Salminen S, Wright AV, Ouwehand A. Lactic Acid Bacteria: Microbiological and Functional Aspects, 2004; 3rd Edition, Marcel Dekker, New York, 1-67. https://doi.org/10.1201/9780824752033.ch1

[7] Parvez S, Malik KA, Ah Kang S, and Kim HY. (2006) Probiotics and their fermented food products are beneficial for health. 2006; J. Appl. Microbiol., 100, 1171–1185.

[8] Steinkraus KH. Fermentations in World Food Processing. Comprehensive Reviews in Food Science and Food Safety, 2002; 1, 23-32. http://dx.doi.org/10.1111/j.1541-4337.2002.tb00004.x

[9] Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. Taxonomic Study of Lancefield Streptococcal Groups C, G, and L (Streptococcus dysgalactiae) and Proposal of S. dysgalactiae subsp. equisimilis subsp. nov. 1996; volume 26, issue 3.

[10] Oladipo IC, Sanni A, Swarnakar S. Phenotypic and genomic characterization of Enterococcus Species from some Nigerian fermented foods. 2013; Food Biotechnology, 27 (1): 39-53. http://dx.doi.org/10.1080/08905436.2012.755627

[11] Versalovic J, Brown DP, Grealish P, Staver MJ, Characterization of the macromolecular synthesis (MMS) operon from Listeria monocytogenes (1994) https://doi.org/10.1016/0378-1119(94)90649-1 Volume 151, Issues 1–2 Pages 1-345

[12] Oladipo IC, Sanni A I, Chakraborty W, Chakravorty S, Jana S, Rudra DS, Gacchui R, Swarnakar S. Technological properties of strains of Enterococcus gallinarum isolated from selected Nigerian traditional fermented foods. 2015; Malaysian Journal of Microbiology 11 (1): 1-13.

[13] Tamang JP, Watanabe K, Holzapfel WH. Diversity of microorganisms in global fermented foods and beverages. 2016; Front. Microbiol., 7, 377.

[14] Muhialdin BJ, Zawawi N, Abdull Razis A F, Bakar J, Zarei M. Antiviral activity of fermented foods and their probiotics bacteria towards respiratory and alimentary tracts viruses. 2021; Food Control, 127:108-140.

[15] Vanniyasingam J, Kapilan R, Vasantharuba S. Isolation and characterization of potential probiotic lactic acid bacteria isolated from cow milk and milk products. 2019; AGRIEAST 2019 13(1): 32-4.

[16] Bintsis TJJ BM. Lactic acid bacteria: their applications in foods. Journal of Bacteriology and Mycology, 2018; 6(2): 89-94.

[17] Kuleshov KV, Margos G, Fingerle V, Koetsveld J, Goptar IA, Markelov ML, Kolyasnikova NM, Sarksyan DS, Kirdyashkina NP, Shipulin GA, Hovius JW, Platonov AE. Whole genome sequencing of Borrelia miyamotoi isolate 2020; Izh-4: reference for a complex bacterial genome. BMC Genomics. 621(1):16.

[18] Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W. Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. 2021; Front Bioeng Biotechnol. 12;9: 612285. doi: 10.3389/fbioe.2021.612285. PMID: 34055755; PMCID: PMC8149962.

[19] Zheng J, Wittouck S, Salvetti E, Franz C, Harris H, Mattarelli P. 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. 2020; Int. J. Syst. Evol. Microbiol. 70 2782–2858. 10.1099/ijsem.0.004107.

[20] Oladipo IC. and Ogunleke OB. Probiotics and Human Health: 2023; An Overview. Journal of Nutrition Food Science and Technology 4(2): 1-8.

[21] Romero-Luna HE, Peredo-Lovillo A, Hernández-Mendoza A. Probiotic Potential of Lactobacillus paracasei CT12 Isolated from Water Kefir Grains. 2020; (Tibicos). Curr Microbiol 77, 2584–2592. https://doi.org/10.1007/s00284-020-02016-0

[22] Olanbiwoninu AA, Odunfa SA. Microbial interaction in selected fermented vegetable condiments in Nigeria. 2020; International Food Research Journal 25(1): 439-455.

[23] Yudha EP, Jamsari Y, Yuherman Y, Melia S, Purwati E. Molecular identification of lactic acid bacteria from "budu" of West Sumatera, Indonesia, as a potential probiotic. 2021; AACL Bioflux, Volume 14, Issue 5. http://www.bioflux.com.ro/aacl

[24] Iqbal MZ. Probiotics and their beneficial effects against various diseases. 2014; Pakistan journal of pharmaceutical sciences, (2) 54-65

[25] Mulaw G, Tessema T, Muleta D, Tesfaye A. In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. 2019; Int J Microbiol.; (11):7179514.

[26] David AAD, Orukotan AA, Mohammed SSD. Conventional and molecular characterization of selected lactic acid bacteria from fermented corn gruel (ogi) and fermented milk (NONO). 2019; Science World Journal Vol. 14(No 4).

[27] Huligere SS, Chandana Kumari VB, Alqadi T, Kumar S, Cull CA, Amachawadi RG, Ramu R. 2023; Isolation and characterization of lactic acid bacteria with potential probiotic activity and further investigation of their activity by α-amylase and α-glucosidase inhibitions of fermented batters. 2023; Front. Microbiol. 13:1042263. doi: 10.3389/fmicb.2022.1042263.