ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

Molecular Characterization of Lactic Acid Producing Bacteria Isolated from Tiger nut (*Cyperus esculentus* L*)* tuber and Soybean (*Glycine max* L*)* seeds

Abu, M. A. *, Amakoromo, E. R. and Eruteya, O. C.

Affiliation: Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.

*Correspondence author

DOI: **https://doi.org/10.51584/IJRIAS.2023.8431**

Received: 30 December 2022; Accepted: 12 January 2023; Published: 19 May 2023

Abstract: Lactic acid-producing bacteria (LAB) are a diverse group of bacteria which play a crucial role in fermentation processes. They produce lactic acid a primary product of fermentation, by exploiting food substances like carbohydrates proteins and lipids, leading to the production of secondary metabolites such as alcohols, aldehydes, acids, esters and Sulphur. The aim of this study was to characterize lactic acid bacterial isolated from tiger nut tubers (*Cyperus esculentus* L) and soybean seeds (*Glycine max* L), using 16S rRNA gene. Lactic acid production was determined by titrimetric method from 0 to 48h. Four of the ten isolates, identified as: *Lactobacillus plantarum* strain AMA2A, *L. fermentum* strain AMA5, *L. buchneri* strain AMA11 and *L. plantarum* strain AMA14 produced percentage lactic acid of 1.27, 0.93, 0.86 and 0.92 respectively after 48h. This study has demonstrated that the lactic acid producing bacteria namely: *Lactobacillus plantarum* AMA2A, *Lactobacillus fermentum* AMA5, *Lactobacillus buchneri* AMA11 and *Lactobacillus plantarum* AMA14 could be isolated from tiger nut and soybean since these substrates contain sufficient ingredients or nutrients which serve as enabling environments for their proliferation

Keywords: Characterization, Fermentation, Lactic acid, Molecular, Soybean, Tiger nut.

I. Introduction

Lactic acid bacteria (LAB) have been linked with food fermentations right from ancient times due to their positive influence on the organoleptic and nutritional property of foods. Their presence in food helps to extend the shelf-life of the food [1]. The antimicrobial peptides and bacteriocins produced by these organisms make them promising in both food, pharmaceutical and other allied industries. Bacteriocins have massive potential in the bio-preservation of a diversity of foods, when used alone as a method of preservation, or in combination with other methods of preservation, regarded as hurdle technology [2]. The antimicrobial activity of many bacteriocins has helped so much in dealing with pathogenic food-borne organisms especially *Listeria monocytogenes* a sturdy pathogen commonly reported to contaminate ready-to-eat refrigerated food products [2].

Lactic acid bacteria (LAB) are known for their ability to produce lactic acid. Their presence in foods plays a pivotal role in of fermentation processes. They make use of carbohydrates to produce lactic acid as primary product of fermentation. They equally utilize lipids and proteins leading to the production of secondary metabolites such as aldehydes, alcohols, esters, acids, and Sulphur compounds, with very important role in food, clinical and agricultural applications [3].

Lactobacillus, Leuconostoc, Pediococcus and *Streptococcus* are common genera belonging to the group of lactic acid bacteria. *Dolosigranulum, Carnobacterium, Aerococcus, Alloiococcus, Enterococcus, Globicatella, Lactococcus* and *Weissella* are included in the new genera following recent taxonomic standard. In fact, now, it is very difficult to establish a clear demarcation between beneficial and dangerous species, because traits that cause serious problems are more connected to strain than to species. However, Lactobacilli and Lactococci are generally regarded as safe (GRAS) organisms [4]. LABS are the most comprehensively studied microorganisms for the fermentation of milk. The bacteria cause fermentation in milk either spontaneously or as inoculated starter cultures [5, 6]. Milk itself is known to be one of the natural habitats of LAB and the most important property of LAB is their ability to acidify milk, generating flavour, texture, and mild acidic taste as seen in yoghurt and cheese [7-11]. Milk and fermented milk products are not only habitats for LAB; they are equally favourable substrates for the growth of spoilage microorganisms. The acid producing ability of LAB gives them a preservative property due to acidification of the milk and milk products, thereby protecting the substrates against the proliferation of spoilage and pathogenic microorganisms. Lactic acid bacteria also release antimicrobial metabolites, bacteriocins which in combination with acid are of immense importance in food preservation, and are considered as safe natural preservatives [11, 12].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

Tiger nut (*Cyperus esculentus* L) is an underutilized crop of the family Cyperaceae, which produces somewhat spherical tubers underneath [13]. It is consumed globally especially in countries such as Spain, as well as in the Arabian Peninsula. It is widely consumed in Africa, particularly Nigeria where its milk is extracted to form 'kunu Aya' a common delicious beverage in the northern part of the country [14].

Soybean (*Glycine max L*) is an important leguminous plant found globally. It has unique functional health benefits, good nutritional value, chemical composition and industrial applications. It is eaten as a processed product because its raw form contains anti-nutritional factors. The milk extracted from soybeans (i.e., soymilk) is very important in human nutrition due to its high protein content [15].

Milk from plant sources like tiger nut and soybean though undervalued and utilized in the ancient times, is key component in African diets, and recently, researchers have shown strong interest in these milk sources due to their high dietary values and economic potentials. The milk from these substrates could be fermented into different products such as beverages, cheeses and other milk products by fermentation with lactic acid bacteria (LAB). The aim of this study was to characterize lactic acid producing bacteria isolated from tiger nut and soybean, using molecular methods.

II. Materials and methods

2.1 Sample Collection and Processing

Dry tiger nut (*Cyperus esculentus* L*)* tubers and soybean (*Glycine max* L) seeds were purchased from Choba Junction Market, Rivers State. The samples were taken in sterile nylon bags to the Department of Microbiology Laboratory, University of Port Harcourt for analysis. The dry yellow tiger nut tubers and the soybean seeds were separately crushed manually using sterile mortar and pestle, followed by soaking overnight. Ten-fold serial dilution was carried out followed by the duplicate plating of 0.1 ml aliquot from appropriate dilutions on De Mann Rogosa and Sharpe agar (MRS) using spread plate method. Plates were incubated at 37ºC for 48 h [16]. Distinct colonies from the various plates were picked and sub-cultured on MRS agar to obtain pure isolates. The isolates were subjected to biochemical tests after which they were preserved in slants for further identification.

2.2 Production of lactic acid by the lactic acid bacterial isolates

The lactic acid content of tiger nut and soymilk blend inoculated with the lactic acid bacterial isolates was determined using titratable acidity method by titrating 10 ml tiger nut and soybean milk blend against 2.5 M sodium hydroxide (NaOH), using phenolphthalein indicator [17]. The acidity was calculated using the formula:

$$\text{Acidity} = \frac{volume\ of\ 0.1N\ NaOH\ used\ x\ 0.2\ x\ 6\ x\ 100}{10\ x\ volume\ of\ sample\ taken}$$

2.3 Bacteriocin producing ability of the lactic acid bacterial isolates.

For bacteriocin production, the isolates were revamped by sub-culturing in MRS broth (Hi Media Laboratory, Pvt Ltd, India) (pH 6.5), containing 1% inoculum, maintained at optimized culture condition for 48 h. After incubation, cells were removed from the culture medium by centrifugation at 1500rpm for15 min at 4ºC. The cell free supernatant was adjusted to pH 6.5 using 1mol/l NaOH and was used as crude bacteriocin.

The bacteriocin activity was measured by standard well diffusion assay [18]. The antimicrobial activity of the purified bacteriocin was carried out by using it against the indicator strains, *Enterococcus* sp. strain 104 and *Listeria innocua* strain 2865 by agar well diffusion method [19]. One hundred microlitres (100µl) of the purified bacteriocin was added in 10mm wells on Mueller Hinton agar plates previously spread with 100 µl suspension of each indicator strain containing 2×10^8 cfu/ml and the plates were incubated for 48 h [20].

2.4 Molecular characterization of the lactic acid bacterial isolates

The four LAB isolates were sub-cultured on MRS medium and incubated at 30°C for 48-72 h, following their morphological, bacteriocin and lactic acid production characteristics. The DNAs of the isolates were extracted and purified using an Invisorb Spin DNA Extraction kit, according to the manufacturers' instructions [21]**.** Fragments of the 16S rRNA genes of each bacterial isolate were independently amplified using the eubacteria universal primers rD1 (5′-AGA GTT TGA TCC TGG CT C AG-3′) and fD1 (5′-AAG GAG GTG ATC CAG CC-3′) [22]. For amplification of 16S rDNA genes of each bacterial isolate, PCR reaction mixtures (50 μl) containing 1μl of the extracted DNA, 5 μl dNTPs, 1 μl of each of the primers rD1 and fD1, 1 μl of *Taq* DNA polymerase (Fermentas, St. Leon-Rot, Germany), and 5 μl PCR buffer. To the content, 50 μl reverse osmosis purified water was added. The temperature of the reaction for initial denaturation step was at 95°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, primer annealing at 51°C for 30 sec, and primer extension at 72°C for 60 sec with a final extension at 72°C for 60 sec [23].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

After running the PCR, the amplicons of LABs were separated by gel electrophoresis using 3% Agarose gel and 1 μL loading dye with 5 μL PCR products and stained with ethidium bromide for gel documentation.

2.5 16S rRNA gene sequence analysis.

After blasting the 16S rRNA sequence, the four lactic acid bacterial strains AMA2A, AMA5, AMA11 and AMA14 were clustered in the genus *Lactobacillus* with 99% similarity among their 16S rDNA gene sequence. Sequence identification was investigated by BLAST n in NCBI search to identify sequence similarity with all available 16S sequence in GeneBank [24].

III. Results

3.1 Physiological and biochemical characteristics of the lactic acid bacterial isolates.

The results of the physiological and biochemical characterization of the LAB isolated from tiger nut tuber and soybean seed is presented in Table 1. Out of the ten (10) lactic acid bacterial isolates, six (6) were isolated from tiger nut and four (4) from soybean. From the result, eight (8) isolates were identified as *Lactobacillus* spp. and two (2) isolates, AMA2b and AMA6 were identified as *Streptococcus* spp. All the isolates were Gram positive and catalase negative.

Table1: Physiological and biochemical characteristics of the lactic acid bacterial isolates from tiger nut and soybean samples.

Isolate	Source of Isolate	Gram's reaction	Oxidate	Catalase	Indole	Citrate	Motility	MR	VP	Slant	Butt	Gas	H2S	Glucose	Sucrose	Maltose	Lactose	Presumptive organisms
AMA2A	TN	+ve rods	-ve	-ve	+ve	+ve	-ve	+ve	-ve	A	A	+ve	+ve	A/G	A/G	A/G	A/G	*Lactobacillus plantarum*
AMA5	TN	+ve rods	-ve	-ve	+ve	+e	-ve	+ve	-ve	A	A	-ve	-ve	A/G	A/G	A/G	A/G	*Lactobacillus fermentum*
AMA11	SB	+ve rods	-ve	-ve	+ve	+ve	-ve	+ve	-ve	A	A	+ve	+ve	A/G	A/G	A/G	A/G	*Lactobacillus buchneri*
AMA14	SB	+ve rods	-ve	-ve	+ve	+ve	-ve	+ve	-ve	A	A	+ve	+ve	A/G	A/G	A/G	A/G	*Lactobacillus plantarum*

Key: MR = methyl red, VP, Voges Proskauer, H_2S = hydrogen sulphide, (+) = positive, (-) = negative, A/G = Acid and Gas, A= Acid, K= Alkaline**.** TN= tiger nut, SB= soybean

3.2 Production of lactic acid by the lactic acid bacterial isolates

The Lactic acid bacteria (LAB) isolated from tiger nut and soybean, were checked for their ability to produce lactic acid for a period of 0 - 48 h of fermentation as shown in Figure 1. It was observed that at 0 h, no lactic acid was produced by the LAB suggesting that the isolates were at the lag phase of growth. The lowest percentage of lactic acid (0.25%) was produced by isolate 13 at 24 h and the highest (1.27%) was produced by isolate 2A (*Lactobacillus plantarum*) at 48 h. The lactic acid produced by the LAB isolates had the following ranges: *Lactobacillus plantarum* strain AMA2A (0.97 to 1.27%), *L. fermentum* strain AMA5 (0.69 to 0.93%), *L. buchneri* strain AMA11(0.65 to 0.86%) and *L. plantarum* strain AMA14 (0.60 to 0.92%).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

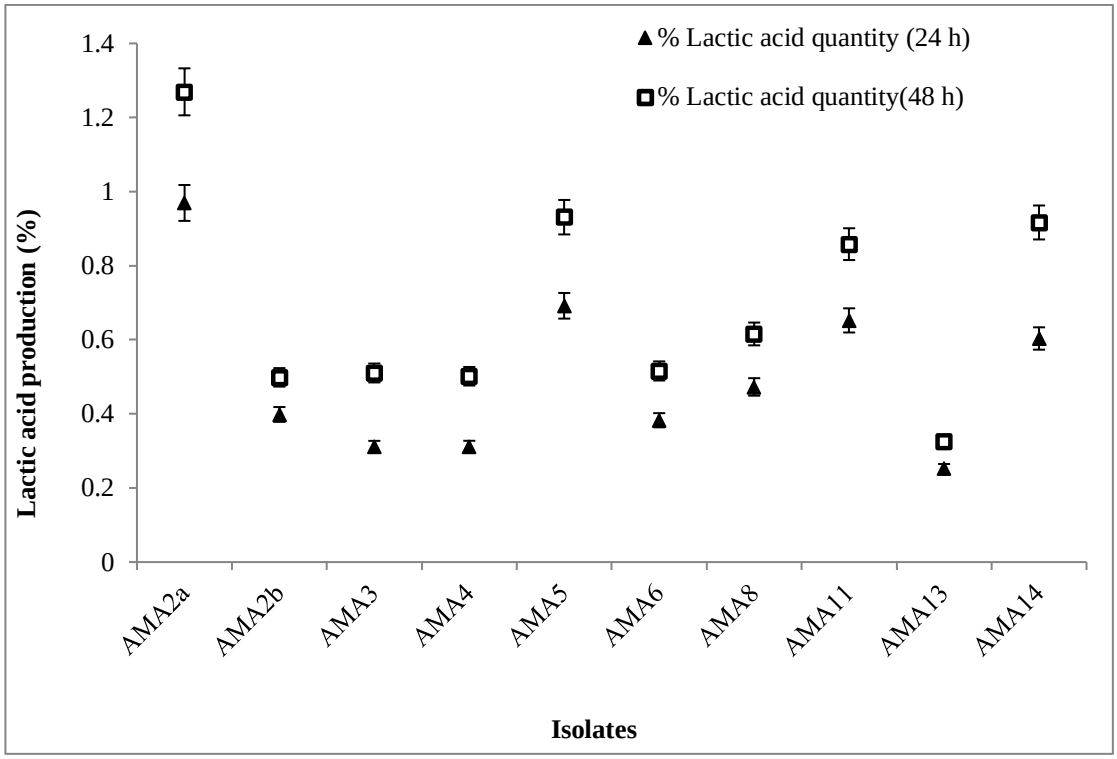

Figure 1: Production of lactic acid by lactic acid bacterial isolates at 0-, 24- and 48-hour intervals using tiger nut and soybean substrates

3.3 Bacteriocin producing ability of the lactic acid bacterial isolates

The bacteriocin activity of the various lactic acid bacteria against *Enterococcus* sp. Strain 104 and *Listeria innocua* strain 2865 was carried out as presented in Table 2. The result showed that isolate AMA5 gave the highest zone of clearance (14.85 ± 0.02 mm) against *Enterococcus* sp. strain 104, whereas isolate AMA2A showed the highest zone of clearance (17.15 ± 0.04 mm) against *Listeria innocua* strain 2865.

Table 2: Activities of bacteriocins from the various lactic acid bacteria against *Enterococcus* sp. Strain 104 and *Listeria innocua* strain 2865

Isolate	Mean Bacteriocin activity (Zone of clearance, mm)	
	Enterococcus sp. strain 104	*Listeria innocua* strain 2865
AMA2A	11±1.56	17.15±0.49
AMA2B	7.75±1.06	12±0.0
AMA3	7.85±0.92	14.9±0.41
AMA4	6.75±0.36	13.5±0.35
AMA5	14.85±0.49	15.55±1.34
AMA6	9.5±1.41	11±0.71
AMA8	7.25±0.0	9.75±0.35
AMA11	10.1±0.14	14.15±0.49
AMA13	10.75±0.35	12.35±0.49
AMA14	10.05±0.64	12.75±0.06

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

3.4 Molecular characterization of the lactic acid bacterial isolates

The PCR amplification image of the16S rRNA gene bands of the LAB isolates AMA11; AMA2A, AMA14, AMA5 and control lanes are presented in Figure 2. The isolates produced the expected 1500 bp for the employed primer. The lactic acid-producing bacteria were identified after sequencing as: *Lactobacillus plantarum* strain AMA2A, *L. fermentum* strain AMA5, *L. buchneri* strain AMA11 and *L. plantarum* strain AMA14.

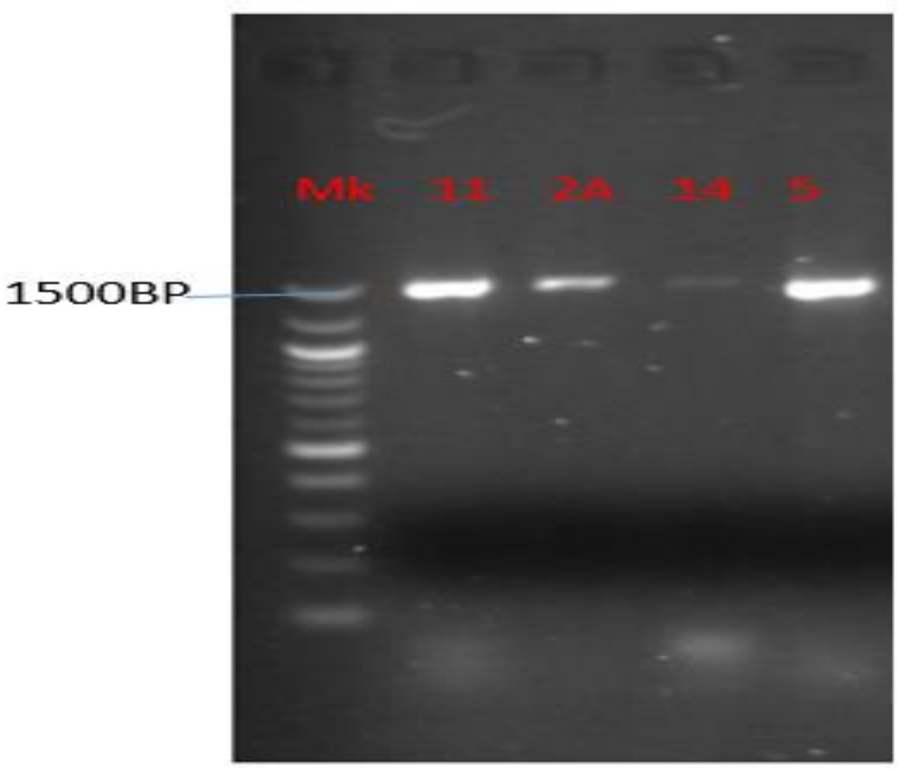

Figure 2: PCR Amplification Image of the16S rRNA gene bands of isolates AMA 11, 2A, 14 and 5 (Lane 1: control; lanes 2, 3, 4 and 5: 16S rRNA of the isolates)

IV. Discussion

The importance of the molecular methods of bacterial identification cannot be overemphasized as they are possibly more accurate than the conventional methods. In recent times, new molecular tools have been used on regular basis for identification of microbes, and this has led to increase in the number of identified bacteria compared to conventional methods of identification [25]. Unlike the conventional cultural and biochemical methods, molecular method is fast, rapid and less stressful; also taking into cognizance the fact the results are more consistent and accurate.

The physiological and biochemical characteristics of the lactic acid bacterial isolates were consistent with reports of previous authors [26-29].

The production of lactic acid by the isolates in this study agrees with the findings of [30] that reported lactic acid production to have increased with increase in fermentation time. Lactic acid can be produced through chemical synthesis and microbial fermentation. Fermentation is an effective and attractive method due to the production of lactic acid of high purity [31]. Lactic acid bacteria are used in food preservation and their antimicrobial effect is mainly due to their lactic and organic acid production, which results in lowering the pH of the growth environment [32].

Hydrogen peroxide, SBC-isomers of amino acids, CO_2, acetaldehyde, reuterin and bacteriocin are the antimicrobial compounds Produced by lactic acid bacteria (LAB) [33]. Bacteriocins are ribosomally synthesized antimicrobial peptides that are effective against other bacteria, either of the same species (narrow spectrum) or across genera (broad spectrum).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

The activities of bacteriocins from the various lactic acid bacteria against *Enterococcus* sp. Strain 104 and *Listeria innocua* strain 2865 could be attributed to the preservative effect exerted by LAB, mainly due to the production of organic acids (such as lactic acid) which results in lowered pH [34].

The molecular characterization revealed that the lactic bacteria isolates in this study have also been isolated by other authors using the same substrates (fermented tiger nut drinks and soybean milk) [35, 36].

V. Conclusion

Four out of the ten (10) lactic acid bacterial isolates produced more lactic acid compared to others. The percentage lactic acid produced by the isolates were as follows: AMA2A (1.27%), AMA5 (0.93%), AMA11 (0.86%) and AMA14 (0.92%). The lactic acid bacterial isolated were characterized as *Lactobacillus plantarum* strain AMA2A, *Lactobacillus fermentum* strain AMA5, *Lactobacillus buchneri* strain AMA11 and *Lactobacillus plantarum* strain AMA14 by molecular methods using 16S rRNA gene analysis which is a more rapid and accurate.

Competing interests

Authors have declared that no competing interests exist.

Acknowledgement

The authors sincerely appreciate the analytical and statistical contributions of Dr F.A. Orji of Federal Institute of Industrial Research Oshodi (FIIRO) and Dr. V. Ezebuiro of Regional Centre for Biotechnology and Bioresource, University of Port Harcourt.

References

1. De Vuyst L, Leroy F. Bacteriocins from Lactic Acid Bacteria: Production, Purification and Food Applications. J Mol Microbiol Biotechnol. 2007; 13(4): 194-199.
2. Chen H, Hoover DG. Bacteriocins and their Food Applications. Compr Rev Food Sci Food Safety. 2003; 2 (3): 82-100.
3. Hayek SA, Ibrahim SA. Current Limitations and Challenges with Lactic Acid Bacteria: A Review. Food Nutr Sci. 2013; 4 (5): 73-87.
4. Bintsis T. Lactic acid bacteria: their applications in foods. J Bacteriol Mycol. 2018; 6(2): 89–94.
5. Olson NF. The impact of lactic acid bacteria on cheese flavor. FEMS Microbiol. Lett. 1990; 879(10): 131-147.
6. Urbach G (1995). Contribution of Lactic Acid Bacteria to Flavour Compound Formation in Dairy Products. Int Dairy J. 1971; 5(8): 877-903.
7. Delavenne E, Mounier J, Déniel F, Barbier G, Le Blay G. Biodiversity of Antifungal Lactic Acid Bacteria Isolated from Raw Milk Samples from Cow, Ewe and Goat over One-Year Period. Int J Food Microbiol. 2012; 155(3): 185-190.
8. Wouters JTM, Ayad EHE, Hugenholtz J, Smit G. Microbes from raw milk for fermented dairy products. Int Dairy J. 2002; 12: 91-109.
9. Griffiths, MW, Tellez AM. Lactobacillus helveticus: the proteolytic system. Frontier Microbiol. 2013; 4(30):1-9.
10. Mäyrä-Mäkinen A, Bigret M. Industrial use and production of lactic acid by Lactic acid bacteria. In: Salminen S, von Wright A, Ouwehand AC, editors Lactic acid bacteria: Microbiological and functional aspects, 3rd Ed. Marcel Dekker, New York. 2014; 175–198.
11. Widyastuti RY, Febrisiantosa A. The Role of Lactic Acid Bacteria in Milk Fermentation. J Food Nutr Sci. 2014; 5(4): 435-442.
12. Zacharof MP, Lovitt RW. Bacteriocins produced by Lactic Acid Bacteria: A review Article, Asia-Pacific Chem Biol Environ Eng. Soc. 2012; 2(3): 50-56.
13. Devries F, Feuke T. Chufa (Cyperus esculentusL.) A weedy cultivar or cultivated weed? Econ Bot. 1999; 45: 27- 37.
14. Abaejoh R, Djomdi I, Ndjouenkeu R. Characteristics of tiger nut (Cyperus esculentus) tubers and their performance in the production of a milky drink. J Food Proc. Preserv. 2006; 30: 145-163.
15. Fan MZ, Sauer WC, de Lange CFM. Amino acid digestibility in soybean meal, extruded soybean and full-fat canola for early-weaned pigs. Ani Feed Sci Technol. 1995; 52(3-4): 189-203.
16. Abu MA, Amakoromo ER, Eruteya OC. Production of Lactic Acid by Lactobacillus plantarum strain AMA 2A Using Tiger Nut as a Substrate. Proceedings of the 2nd Biotechnology Society of Nigeria (BSN), South-East Zonal Symposium Abia. 2021:211-214.
17. AOAC. Official Methods of Analysis. 17th ed, The Association of Official Analytical Chemistry, Gaithersburg, MD, USA. 2000.
18. Kang JH, Lee MS. Characterization of a bacteriocin produced by Enterococcus faecium GM-1 isolated from an infant. J Appl Microbiol. 2005; 98 (5): 1169-1176.
19. Tagg J, McGiven AR. Assay System for Bacteriocins. J Appl Microbiol.21(5): 943 – 953..

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue IV April 2023

20. Iqbal, A. Production, purification and characterization of bacteriocins from indigenous clinical staphylococci. Ph.D. Thesis, University of Karachi, Pakistan Research Repository, Higher Education Commission, ID code 1112. 1998.
21. Gevers D, Huys G Swings J. Applicability of rep-PCR fingerprinting for identification of Lactobacillus species, FEMS Microbiology Letters, 2001; 205(1): 31–36.
22. Benson DR, Stephens DW, Clawson ML, Silvester WB. Amplification of 16S rRNA genes from Frankia strains in root nodules of Ceanothus griseus, Coriaria arborea, Coriaria plumosa, Discaria toumatou, and Purshia tridentate. Applied and Environmental Microbiology, 1996; 62(8):2904–2909.
23. Evelyn M. Molecular Characterization and Population Dynamics of Lactic Acid Bacteria during the Fermentation of Sorghum. University of Pretoria. 2009: 1–54.
24. Silva GA, Bernardi TL, Schalker PDC, Menegotto M, Valente P. Rapid DNA extraction by boiling and freeze-thawing without using chemical reagents and purification. Brazil Arch Biol Technol. 2012; 55(2): 319-327.
25. Figure I, Atigui M, Manel Z, Samira A. Biochemical and molecular identification of lactic acid bacteria isolated from camel milk in Tunisia. Emirates J Food Agri. 2015;27(9): 716 – 720.
26. Ngene AC, Onuakor CE, Aguiyi JC, Ifeanyi VO, Ohaegbu CG, Okwuchukwu CP, et al. Screening of some lactic acid bacteria isolated from selected Nigerian fermented foods for vitamin production. Adv Microbiol. 2019; 9: 943-955.
27. Ahmad MS, Zargar MY, Mir SA, Bhat NA, Baba ZA, Kant RH, et al. Morphological and biochemical studies for the identification of Lactobacillus plantarum sp. nov., and Lactobacillus fermentum sp. nov., from municipal waste. J pharmacol. phytochem. 2018;7(5): 1421-1424.
28. Yelnetty A, Purnomo H, Purwadi, MA. Biochemical characteristics of lactic acid bacteria with Proteolytic Activity and Capability as Starter Culture Isolated from Spontaneous Fermented Local Goat Milk. J Natural Sci Res. 2014; 4(10):137-146.
29. Nair PS, Surendran PK. Biochemical characterization of lactic acid bacteria isolated from fish and prawn. J Cult collect. 2005; 4: 48-52.
30. Zhang B, He PJ, Shao LM. Effect of pH and fermentation time on yield and optical purity of lactic acid from kitchen wastes fermentation. Huan Jing Ke Xue 2007; 28(4):881-5.
31. Mujtahid K, Fittriani K, Saeed MA. Lactic acid production from date juice using Lactobacillus casei ATCC 393 in batch fermentation. MATEC Web of Conferences 101. 02002. 2017 DOI:10.1051/matecconf/201710102002.
32. Dheeraj M, Ravinder N, Manoj K, Aarti B. Molecular methods for identification and characterization of lactic acid bacteria: a review. J Digest Dis. 2008; 9 (4): 190-198.
33. Cintas LM, Casaus MP, Herranz C, Nes IF, Hernández PE. Review: bacteriocins of lactic acid bacteria. Food Sci Technol Int. 2001; 7(4): 281-305.
34. Daeschel MA. Antimicrobial substances from lactic acid bacteria for use as food preservatives. J Food Agri Org United Nations. 1989; 4(1): 164-167.
35. Kilau FM, Mohamad NI, Saari N, Sani NA. Isolation, characterization, and identification of lactic acid bacteria from fermented soy sauce. AIP conference proceedings, 2111, 040006 2019; https://doi.org/10.1063/1.5111245
36. Aritonang SN, Roza E, Rossi E, Purwati E. Isolation and identification of Lactic Acid Bacteria from Okara and Evaluation of their potential as candidate probiotics. Pak J Nutr. 2017; 16(8): 618-628.
37. Maduka N, Ire FS, Njoku HO. Fermentation of tiger nut by lactic acid bacteria and tiger nut-milk drink fermentation by lactic acid bacteria as a potential probiotic product. Asia Pac J Sci Technol. 2017; 8 (7): 5167-5172.