Anchor University Journal of Science and Technology (AUJST)
A publication of the Faculty of Science Applied and Health Science, Anchor University Lagos
URL: journal.aul.edu.ng
In AJOL: https://www.ajol.info/index.php/aujst
Vol. 6 No 2, November 2025, Pp. 236-242
ISSN: 2736-0059 (Print); 2736-0067 (Online)

Development of Starter Culture for Milk Fermentation Using Lactic Acid Bacteria Isolated from Deteriorating Apple and Fermented Millet.

[1]Adedayo Majekodunmi Racheal, [2]Akinbo Omotola Bukola [3]Said Olaitan Rukayat, [4]Abdulrahman Muhammad

[1234]Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria.

***Corresponding author Email**:
rukayat.said@kwasu.edu.ng

Submitted 20 September, 2024
Accepted 22 July, 2025

Competing Interests.
The authors declare no competing interests

ABSTRACT

Background: In food fermentation, starter cultures are often used to enhance the sensory qualities, nutritional value, and shelf life of food. **Objectives**: Lactic acid bacteria (LAB) isolated from fermented millet and deteriorating apples were used in the study to produce a starting culture for milk fermentation. **Methods**: Isolation, characterization, and identification of LAB were done using standard microbiological techniques. The isolated LAB were used as starter culture singly and as mixed culture in the fermentation of milk at 28±2 °C for 5 hours. The fermented milk samples were subjected to physicochemical assay and sensory evaluation using approved methods. **Results**: Five *Lactobacillus* species were obtained, the physiochemical parameters of the fermented products were in the range of Moisture content (75.02 to 76.29%), Ash content (1.51 to 1.75%), Total fat (4.35 to 4.67%), Total protein (6.37 to 7.06%), Total titratable acid (0.32 to 0.58%), pH (4.31 to 4.59%) and Nitrogen free extract (10.90 to 13.00%). The sensory property was in the range of 23.4% to 78.0%. **Conclusion**: The study concluded that the isolated LAB could be potentially employed as native fermenters in the milk fermentation process, as well as possible starter cultures in the dairy industry.

Keywords. Deteriorating Apples, Lactic Acid Bacteria, Milk, Organoleptic Properties, and Starter Culture.

1. Introduction

Starter cultures are single or combination formulations of specific strains of microorganisms that, when added at appropriate quantities, have a particular physiological capacity to transform a substrate into a food product with particular properties (Laranjo *et al.*, 2017). The standard starter helps to increase control over the fermentation process and the predictability of its products by adapting to the substrate (Holzapfel *et al.,* 2003). The evolution of traditional food fermentations in developing nations has been propelled by the development, growth, and standardization of microbial starters. Innovation in the construction of equipment appropriate for the sanitary processing of traditional fermented foods under regulated conditions has also been driven by the creation of microbial starter cultures in many developing nations. Lactic acid bacteria have a variety of uses in the food, medicine, and in the fields of agriculture (Bintsis, 2018). Lactic acid bacteria are non-spore-forming, non-respiring, but aerotolerant. These bacteria produce lactic acid as an end product of carbohydrate catabolism and also make organic substances that contribute to the flavor, texture, and aroma that result in unique organoleptic characteristics (Hayek *et al.,* 2019). Lactic acid bacteria have unique nutritional qualities and improved adhesional adaptation traits that allow them to readily flourish in a variety of settings, including the human gut, plants, dairy products, and fermented meals (Bintsis, 2018). Lactic acid bacteria are very helpful in many applications, particularly in the food and dairy industries, because they create organic acids and other metabolites during fermentation that improve food flavor development and prevent spoilage. A natural technique for extending the shelf life and preserving food is bio-preservation, which uses a controlled microbiota. Lactic acid bacteria are used in this process. Thus, in the area of food safety and

https://dx.doi.org/10.4314/aujst.v6i2.6

spoilage, bio-preservation is regarded as one of the numerous qualities derived from lactic acid bacteria. Despite these facts, milk allows the growth of numerous infectious pathogens, putting consumers of milk products at risk.

Fruit peels, bagasse, and olive mill waste are effective in both submerged and solid-state fermentation systems (Agrahari *et al*., 2024). Moraes and Silva (2024) found that coffee processing byproducts enhance flavor and microbial viability in starter cultures. Cereal grains like millet have been used to develop spray-dried starter culture in traditional African fermented foods (Oguntoyinbo and Narbad, 2015). Whey + molasses-based media supported *Lactobacillus lactis* growth with 87% lower cost than commercial media (Alhassan *et al.,* 2024). Thus, limited studies have examined LAB from spoiled fruits and grains such as apple and millet as starter culture for milk-based fermentation; most starter culture comes from fermented milk or commercial strains, not from non-conventional, spoilage–adjacent sources. The purpose of milk fermentation utilizing LAB is to increase the milk's shelf life and retain its nutritious content because of its highly perishable nature. The objectives of the current work were to use LAB isolated from fermented millet and deteriorating apples to produce a starter culture for milk fermentation, thus offering a novel route to starter culture development.

1.1 Research Hypothesis

LAB isolated from deteriorating apple and fermented millet can effectively ferment milk and produce results similar to standard starter cultures.

H_0: LAB from deteriorating apple and millet has no significant effect on milk fermentation

H_1: LAB from deteriorating apple and fermented millet support milk fermentation.

2. Material and Methods

2.1 Sample Collection

Millet grains used in this study were purchased from Oja-Oba market, Akure, Ondo state, Nigeria. Apple fruits were also purchased from Oja-Oba market, Akure, Ondo state, Nigeria. The research analyses were performed at the Microbiology Department, Federal University of Technology, Akure, Ondo State.

2.2 Sample Processing

The Millet grains were physically sorted to remove dirt, stones, broken or moldy grains, and washed. Apple fruits were also thoroughly washed in a series of clean water to remove any debris that might adhere. Before being used, the two types of apple fruits—green and red—were allowed to deteriorate.

2.3 Fermentation

The millet was subjected to spontaneous fermentation at 30˚C ± 2˚C for 72 hours (Rodriguez-Tudela *et al*., 2003). Additionally, the millet water that had fermented was aseptically decanted into a sterile container.

2.4 Preparation of Culture Media

Lactic acid bacteria were isolated and enumerated using De Man, Rogosa, and Sharpe agar (Oxoid, UK) culture media. The manufacturer's instructions were closely followed to prepare the medium (Ogbuile *et al.,* 2007).

2.5 Sample Preparation, Isolation, and Enumeration of Microorganisms

The millet sample was serially diluted in duplicate up to 7-fold (10^{-1} to 10^{-7}). The standard pour plate method was used to isolate lactic acid bacteria (Ogbuile *et al.,* 2007), using De Man Rogosa and Sharpe Agar; the plates were incubated at 37.0°C for 48 hours. The total numbers of viable colonies were determined by counting distinct colonies.

2.6. Characterization and Identification of Isolates

2.6.1 Colonial and Cellular Characteristics of Lactic Acid Bacterial Isolates

The bacterial isolates were characterized based on their colonial and cellular characteristics. The cellular characteristics of the isolates were observed after Gram staining and viewed under the microscope. The bacterial isolates were further identified using conventional biochemical methods (Fawole and Oso, 2007).

2.7 Re-inoculation of LAB Isolates into Fresh Milk

Nunu was prepared using powdered milk as described by (Akabanda *et al.*, 2014) and modified using the isolated organisms as inoculum.

2.8 Physicochemical Analysis and Proximate Analysis

The physicochemical analysis and proximate analysis of the fermented milk were done following standard methods described by the Association of Official Analytical Chemists (AOAC, 2005).

2.9 Organoleptic Assessment of the Fermented Milk

The purpose of this evaluation was to ascertain the taste's quality. According to Cartwright *et al.* (2006), a panel consisting of twenty members conducted this assessment. Taste, color, smell, consistency, and overall acceptability were the attributes taken into consideration.

3. Results

3.1 Bacterial Isolates

A total of five isolates were obtained from the samples. The result is presented in Table 1.

Table 1: Bacterial Isolates

Samples	Isolates ($x10^6$Cfu/ml)
RA	2. 00
GRA	**1.00**
MIL	**2.00**

Keys:

RA = Isolates from red apple, GA = Isolates from green apple, MIL = Isolates from Millet

3.2 Cultural / Morphological Characteristics of Bacterial Isolates

The Gram reaction of the bacterial isolates was observed, in which they all tested positive and were all rod-shaped. The morphological characteristics of the isolates were observed in terms of shape, pigmentation. The colonial morphology of the bacterial isolates is presented in Table 2.

Keys: RA 1 = Isolate from red apple one, RA 2 = Isolates from red apple two, GA =

Table 2: Cultural / Morphological Characteristics of Bacterial Isolates

Isolates	Grams	Shape	Pigmentation
RA1	+ve	Rod	Creamy
RA2	+ve	Rod	Creamy
GA	+ve	Rod	Creamy
MIL 1	+ve	Rod	Creamy
MIL 2	+ve	Rod	Creamy

Isolate from green apple, MIL 1 = Isolate from millet one, MIL 2 = Isolates from millet two; +ve = Positive, -ve = Negative

3.3 Biochemical Characteristics of Bacteria Isolated from Fermented Millet and Deteriorated Apples

All the isolates showed different biochemical properties and morphological features. All isolates were positive for Voges-Proskauer, methyl red, and indole, while they were also negative for catalase and oxidase. Two of the isolates were also positive for citrate (MIL 1 and MIL 2). All the isolates were able to ferment the sugar used, with production of colour change with or without gas production (Table 3).

3.4 pH and Titratable Acidity of Fermented Milk after 24 hours of Storage

The pH of fermented milk is presented in Table 4, where the highest value was recorded from isolates E, while the lowest pH was observed in isolates BC. The titratable acidity of the fermented milk ranges from 0.32 to 0.65%. Sample fermented with A isolates recorded the highest titratable acidity value, while the lowest value was observed in

https://dx.doi.org/10.4314/aujst.v6i2.6

Table 3: Biochemical Characteristics of Bacteria Isolated from Fermented Millet and Deteriorated Apples

Iso-	CAT	INDO	V.P	CIT	OXI	M.R	DIA	P.O
RA1	-	+	+	-	-	+	-	*Lactobacillus* sp
RA 2	-	+	+	-	-	+	+	*Lactobacillus* sp
GA	-	+	+	-	-	+	+	*Lactobacillus* sp
MIL 1	-	+	+	-	-	+	+	*Lactobacillus* sp
MIL 2	-	+	+	-	-	+	+	*Lactobacillus* sp

Keys:

RA1= *Lactobacillus* sp, RA2= *Lactobacillus* sp, GA= *Lactobacillus* sp, MIL 1= *Lactobacillus* sp, MIL 2 = *Lactobacillus* sp, + = Positive , - = Negative , CAT = Catalase, INDO = Indole test, V.P = Voges proskeur test, CIT = Citrate test, OXI = Oxidase test, MR = Metyl red test, P.O = Probable organisms, DIA = Diacetyl

Table 4: pH and Titratable Acidity of Fermented Milk after 24 hours of Storage

Fermented samples	pH	TTA (%)
L1	**4.50±0.01**	**0.65±0.00**
L2	**4.38±0.04**	**0.44±0.01**
L3	**4.51±0.12**	**0.46±0.01**
L4	**4.25±0.06**	**0.43±0.01**
L5	**4.19±0.01**	**0.46±0.07**
L1+L2	**4.38±0.04**	**0.36±0.04**
L3+L4	**4.33±0.09**	**0.58±0.03**
L1+L5	**4.42±0.07**	**0.47±0.04**
L4+L5	**4.31±0.01**	**0.44±0.03**
L2+L3	**4.59±0.02**	**0.32±0.05**
Ctrl +	**4.48±0.03**	**0.38±0.05**
Ctrl -	**4.27±0.06**	**0.48±0.08**

Keys: Red Apple 1= L1, Red Apple 2= L2, Green Apple= L3, Millet 1= L4, Millet 2=L5, Ctrl+= No isolates, Ctrl-= All isolates

isolates BC (Table 4).

3.5 Proximate Composition of Fermented Milk

The proximate composition of fermented milk is presented in Table 5. The moisture content ranges from 75.09 to 76.29 %, where isolates AE were recorded the highest percentage, and BC isolates recorded the lowest. The highest ash content was recorded in isolates AB with 1.75 %, while the lowest was recorded in isolates BC with 1.51%. The highest fat content was recorded in isolates DE, while the lowest value was recorded in AB. The Protein content recorded ranges from 6.35% to 7.06%, where AB had the highest value and AE had the lowest value. Also, the carbohydrate content ranges from 10.90 to 13%, where BC isolates were recorded as the highest value and CD was recorded lowest.

3.6 Sensory Evaluation of Fermented Milk

As shown in Table 6. The highest percentage for the sensory evaluation test was recorded from the positive control sample, while the negative control recorded the lowest.

4.1 Discussion

Lactic acid bacteria have been identified as one of the primary organisms utilized in milk fermentation; this finding may be because of their well-known fermentative capacity, similar to the work of Panesar *et al. (*2011). In general, the dominant LAB were those with a rod shape. A similar observation was noted by Yelnetty *et al. (*2020). They found that 61.5% of the LAB strains isolated from locally fermented goat milk have a rod shape.

According to the work of Alkema *et al*. (2016), LAB are gram-positive rods, mostly catalase and oxidase-negative. When developing starter cultures for fermented milk products, rapid acidification is a top priority. The fermenting medium's ability to suppress the growth of pathogens like *Salmonella* sp. has been found to increase with the rate at which pH decreases. According to Singh *et al*. (2012), millet proteins are a good source of essential amino acids, so the presence of protein in fermented milk makes it healthier to consume. It has been noted that the percentage of fat content in yoghurt is important because it enhances the yoghurt's texture, appearance, taste, and flavor (Oladipo *et al.,* 2014).

Table 5: Proximate Composition of Fermented Milk

Moisture%	Ash %	Fat %	Protein %	NFE %
L1+L275.52±1.32	1.75±0.19	4.35±0.58	7.06±0.06	11.32±0.92
L3+L4 76.02±1.05	1.54±0.23	4.59±0.26	6.90±0.44	10.90±1.63
L1+L5 76.29±1.15	1.52±0.21	4.52±0.51	6.35±0.33	11.45±0.65
L4+L5 75.13±0.05	1.59±0.16	4.67±0.51	6.79±0.05	11.83±0.67
L2+L3 75.09±0.60	1.51±0.08	4.37±0.06	6.37±0.06	13.00±0.12

Keys: Red Apple = L1, Red Apple 2=L2, Green Apple=L3, Millet=L4, Millet=L5, TTA = Titratable Acidity, NFE= Carbohydrate, Note data are means of replicate plus or minus standard deviation.

https://dx.doi.org/10.4314/aujst.v6i2.6

Table 6: Sensory Evaluation of Fermented Milk

Fermented samples	**Sensory Evaluation (%)**
A	65.6
B	64.0
C	73.6
D	**76.8**
E	45.0
AB	**66.4**
CD	**75.6**
AE	**50.4**
DE	49.2
BC	**64.4**
Ctrl +	**78.0**
Ctrl -	**23.4**

Keys: Red Apple 1= B, Red Apple 2= E, Green Apple = A, Millet1 = C, Millet 2 = D, Ctrl += Positive control, Ctrl - = Negative control

USDA (2001 states that yoghurt samples with more than 3.25 % fat should be labeled as yoghurt; those with 0.5–2 % fat should be labeled as low-fat yoghurt; and those with less than 0.5 % fat should be labeled as non-fat yoghurt. Yogurt should have a moisture level of less than 84 %. Higher moisture content has an impact on mouth feel and texture (Igbabul, 2014). The importance of milk moisture content is that high moisture content indicates high water activity, which promotes microbial development and shortens the milk sample's shelf life (Ajai *et al,* 2012).

5. Conclusion

According to this study, lactic acid bacteria that were isolated from fermented millet showed promising technological qualities and were later employed as milk fermentation starter cultures. Yogurt with desired consumer sensory qualities could be produced using LAB species starter cultures, either alone or in combination. Therefore, the product's safety and customer acceptance will be enhanced by further research and the use of these cultures in commercial yoghurt manufacturing.

References

A0AC (2005). Official methods of analysis. Association of Analytical Chemists, 15th edition. Horwitz, W. and Latimer, G.W. (Eds).AOAC International, Maryland-USA.

Agrahari, S., Ghosh, R., and Singh, R. (2024). Agro-industrial residues as fermentation media. *Fermentation,* 10(2), 112. https// doi.org/10.3390/fermentation10020112

Akabanda, F., Owusu-Kwarteng, J., Ta-no-Debrah, K., Glover, R. L. K., Nielsen, D., and Jespersen, L. (2013). Taxonomic and Molecular characterization of lactic acid bacteria and yeasts in nunu, a Ghanaian fermented milk product. *Food Microbiology*. 34 (2), 277–283.

Alhassan, M., Abol-Monafi, A., and Samat, A. (2024). Design of an agro-industrial by-product-based medium for sustainable development of microbial starter cultures. *Scientific Reports*, 14(1), 12561. https:// doi.org/10.1038/s41598-024-60009-9

Alkema, W., Boekhorst, J., Wels, M., and Hijum., S.A.F.T. (2016). Microbial bioinformatics for food safety and production. *Brief. Bioinform.* 17, 283–292.

Bintsis, T. (2018).*Lactic acid bacteria starter cultures: An update in their metabolism and genetics. Aims Microbiology* 4, 665–68 4.

Fawole, M.O. and Oso, B.A. (2OO7). Characterization of bacteria: Laboratory Manual of Microbiology. Revised Edition. Spectrum book limited 24-33.

Hayek, S.A., Gyawali, R., Aljaloud, S.O., Krastanov, A. and Ibrahim, S.A. (2019). *Cultivation media for lactic acid bacteria used in dairy products. Journal of Dairy Resources.* 86, 490–502.

Holzapfel, W.H. (2003). Use of Starter Cultures in Fermentation on a Household Scale. *Food Control,* 8, 241-258. https://doi.org/10.1016/ S0956-7135 (97)00017-0.

Igbabul, B., Hiikyaa, O., and Amove, J. (2014).

Effect of Fermentation on the Proximate Composition and Functional Properties of Mahogany Beans (*Afzelia africana*) Flour. *Current Resource Nutrition Food Science*, 2(1), 23-56.

Laranjo, M., Elias, M., and Fraqueza, M.J. (2017). The Use of Starter Cultures in Traditional Meat Products. *Journal of Food Quality, Article ID:* 9546026.

Moraes, A.M., and Silva, C.F. (2024). Use of coffee processing by-products. *Microorganisms*, 12(1), 55. https//doi.org/10.3390/microorganisms12010055

Ogbuile, J.N., Ogueke, C.C., and Nwanebe, F.C. (2007). Antibacterial properties of *Uvariachamae, Congrone malatifolium, Garcinia kola, Vernonia amygdalina,* and *Aframomium melegueta*. *African Journal of Biotechnology*. 6:1549-1553.

Oguntoyinbo, F.A., and Narbad, A. (2015). Molecular ecological and health-promoting potential of fermented cereal-based products. *Journal of Food Microbiology,* 47, 21- 31. https//doi.org/10.1016/j.fm.2014.12.003

Oladipo, O.C., Atolagbe, O.O., and Adetiba, T.M. (2014). Nutritional Evaluation and Microbiological Analysis Of Yoghurt Produced From Full Cream Milk, Tiger-Nut Milk, Skimmed Milk, and Fresh Cow Milk. *Pensee Journal*. 2 (6), 23-69.

Panesar, P.S. (2011). Fermented Dairy Products: Starter Cultures and Potential Nutritional Benefits. *Food Nutrition Science*. 2(1):47-51.

Rodriguez-Tudela, J. L., Chryssanthou, M., Barchiessi, F., Bille, J., Moore, C., Richardson, M., and Verweij, P.E. (2003). Method for the Determination of Minimum Inhibitory Concentration (MIC) by Broth Dilution of Fermentative Yeasts. *Clinical Microbiology and Infection*. 1-5.

Singh, K.P., Mishra, A. and Mishra, H.N. (2012). Fuzzy Analysis of Sensory Attributes of Bread Prepared from Millet-Based Composite Flours. LWT—*Food Science and Technology.* 48: 276–82.

USDA (2001). United States Department of Agriculture: Specifications for Yogurt, Non-Fat Yogurt, and Low-Fat Yogurt. 1-3.

Yelnetty, A., Purwadi, and Trina E.T. (2020). Indigenous Lactic Acid Bacteria Isolated from Spontaneously Fermented Goat Meat as Potential Probiotics. *Pakistan Journal of Biological Sciences.* 1028-8880.

Acknowledgement

We sincerely appreciate the Departments of Microbiology, Federal University of Technology, Akure, Ondo State, and also Kwara State University, Malete, for the success of this work.