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Bacteriological assessment of lactic acid bacteria in the last stage effluents from cassava and Ogi production

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Abstract

The objective of this work was to isolate and characterize lactic acid bacteria in the last stage effluents of Cassava (Fufu) and Ogi production. A total of two hundred last stage effluents (consisting of 100 each from Ogi and cassava during processing), were randomly collected in a 10 ml wide-mouthed sterile sample container from processing homes in Ezza-North L.G.A for 20 days. The collected samples were aseptically analyzed using standard microbiological methods. The results showed that the lactic acid bacterial counts of the last stage effluents generated from ogi and cassava (fufu) production range from 7.0×10^4 to 8.8×10^5. Ogi effluents accounted for 113 LAB comprising *Lactiplantibacillus argentoratensis* (n=20), *Lactococcuslactis* (n=24), *Lactiplantibacillus plantarum* (n=24), *Leuconostoc pseudomesenteroides* (n=14), *Leuconostoc mesenteroides* (*n*=16) *Pediococcus pentosaceus* (*n*= 15). Fufu effluents accounted for 122 LAB comprising *Lactiplantibacillus argentoratensis* (n=25), *Lactococcus lactis* (n=26), *Lactiplantibacillus plantarum* (n=19), *Leuconostoc pseudomesenteroides* (n=20), *Leuconostoc mesenteroides* (*n*=15) *Pediococcus pentosaceus* (*n*= 17). It also revealed that the last stage effluents of Ogi and fufu are suitable sources of new LAB such as *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus* which have not been identified in related studies from Nigeria. Therefore an *in vivo* and *in vitro* probiotic characteristic of these potential *Lactobacillus* strains is encouraged.

Keywords: Cassava, Ogi, effluents, LAB

Introduction

Lactic acid bacteria (LAB) are a diverse category of probiotic bacteria, with Lactobacilli being the most prolific and non-pathogenic strain (Ayodeji *et al.*, 2017; Olatunde *et al.*, 2018) [22, 21]. In recent decades, lactic acid bacteria (LAB) have garnered significant interest owing to the health benefits associated with specific strains. They are utilized in a diverse array of fermented foods and play an essential role in food processing and spontaneous fermentation (Ngene *et al.*, 2019; Orji *et al.*, 2021; Ohaegbu *et al.*, 2022) [23, 1, 24].

Growing consumer awareness of a healthy lifestyle has led to an always rising demand for food products with versatile health benefits including, for example, food items containing live microbial cultures which, when consumed by people or animals (In the form of dehydrated cells or fermented products), can favorably affect their health by improving the properties of the original microbiota (Ayodeji *et al.*, 2017) [22].

One of the most effective methods for obtaining genetically stable and practical bacterial strains has always been the isolation and screening of microorganisms from natural sources (Adnan and Tan 2006). For example, it is generally understood that spontaneously fermented foods containing mixed cultures can be used to produce bacterial strains. Indeed, in many situations, such microbes exhibit stable features, and they are especially capable of enduring stressors due to the complex environment from which they were isolated. Lactic acid bacteria (LAB) emerged as an industrial important bacteria in food microbiology with the above-mentioned qualities. LAB has been widely investigated diary, dietary fibers, and cereal-based products (Lamsal and Faubion, 2009; Sanni *et al*. 2013; Ngene *et al.*, 2019; Ayodeji *et al.*, 2017; Olatunde *et al.*, 2018; Ohaegbu *et al.*, 2022) [19, 20, 23, 22, 21, 24].

A variety of fermented dietary fibers, such as cassava, is a very important crop in many African, Asian, and South American countries, and according to Food and Agriculture Organization data, it is the fourth most consumed crop globally (Ferraro 2016) [18]. In 2010, Nigeria produced 37.5 million tons of cassava, making it the world's largest producer (Ishola *et al*., 2013) [17].

Indeed, many traditional Nigerian dishes are based on and/or derived from cassava, generating wastes in various forms, both solid and liquid.

According to the research, numerous cassava-derived food products and by-products can be good sources of LAB (Anyogu *et al.*, 2014; Avancini *et al.*, 2007; Banwo *et al*. 2012; Ouoba *et al*., 2010; WilfridPadonou *et al*. 2009) [16, 14, 15, 13, 12]. Fufu is a traditional cassava-based Nigerian cuisine that is produced by fermentation procedures, as shown in Fig 1a (Anyogu *et al*. 2014) [16]; thus, both products and/or by-products can be used for LAB isolation.

Furthermore, cereals such as maize are nutritionally significant diets in Sub-Saharan Africa because they are rich in carbohydrates, proteins, lipids, and vitamins. (Banwo *et al*., 2023; Kayitesi *et al*., 2023) [11, 10].

Ogi is a traditional fermented cereal gruel derived from maize. It was traditionally changed by submerged fermentation into a fermented porridge called Ogi (as shown in fig 1b), which is commonly consumed in Nigeria and West Africa (Banwo *et al.*, 2023) [11]. It is an important part of the diet for both children and adults; it is commonly used as a weaning food for infants and is the ideal food for the convalescent and elderly. Ogi have a smooth texture like hot blancmange and a sour flavor similar to yoghurt. Its color is determined by the cereal used, with cream or milk white for maize, reddish brown for sorghum, and dirty gray for millet. Ogi is given different names based on its consistency and form when ingested, such as akamu, agidi (eko), and koko (Kayitesi *et al*., 2023) [10]. Fermenting cereals with probiotic microbes has nutritional benefits such as higher digestibility, improved amino acid content, reduced energy requirement for heating due to the metabolism of complex compounds by the fermenting organisms, and improved organoleptic qualities (Nyanzi and Jooste, 2012; Chaves-Lopez *et al.*, 2023) [8, 9]. Several authors have reported LAB isolates from effluents of fermented ogi and fufu product (Ayodeji *et al.*, 2017; Olatunde *et al.*, 2018) [22, 21]. However, certain LAB species unidentified or not well documented may exist in final processing Ogi and fufu effluent that are typically thrown away. Continuous bacteriological assessment of the last effluent may provide insight into new species presence in the effluent.

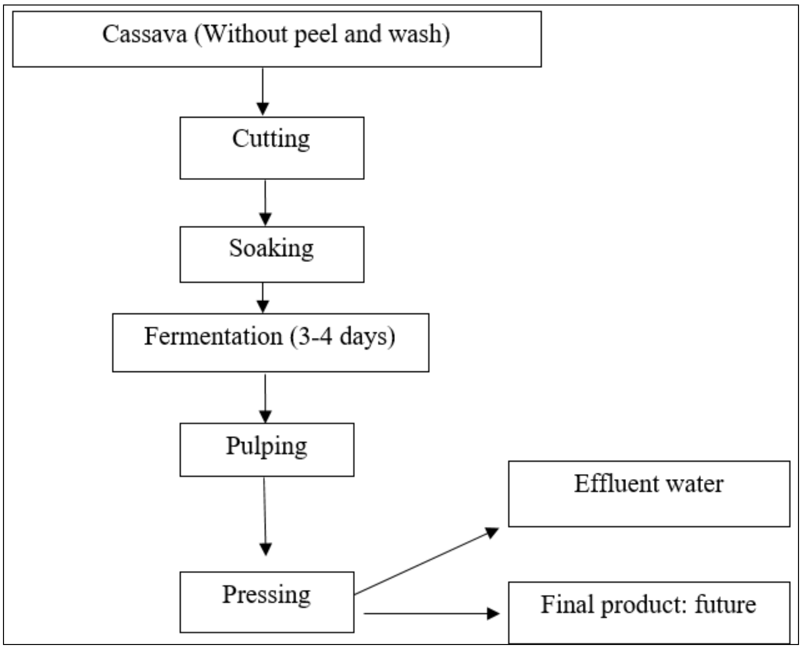
Materials and Methods

Study Area

This study was carried out in Ezza-North local government of Ebonyi state. Ezza-North is one of the thirteen local government areas in Ebonyi state with headquarters at Ebiaji. Ezza-North local government area has a landmass of 305km². It is occupied primarily by the Igbo farmers and had a population of 145,619 at the 2006 census. The Global positioning System (GPS) coordinate of Ezza North, Nigeria is Latitude 6.2708 and Longitude 7. 9713. Ezza-North Local Government Area is located in the Ebonyi State Central senatorial zone of Ebonyi State. It is bordered in the north by Ohaukwu LGA and Ebonyi LGA, in the south by Ezza-south LGA, in the east by Abakaliki LGA and in the west by Ishielu LGA. Ezza-North local government area is made up of eleven traditionally recognized communities namely: Ndiaguazu-Umuoghara, Okposi-Umuoghara, Orizor, Omege/Oshiegbe-Umuezeokoha, Umuezeoka, Nkomoro, Ekka, Iyere, Ogboji and Amaudo/Amawula (Nwele and Nwaorgu, 2017) [7].

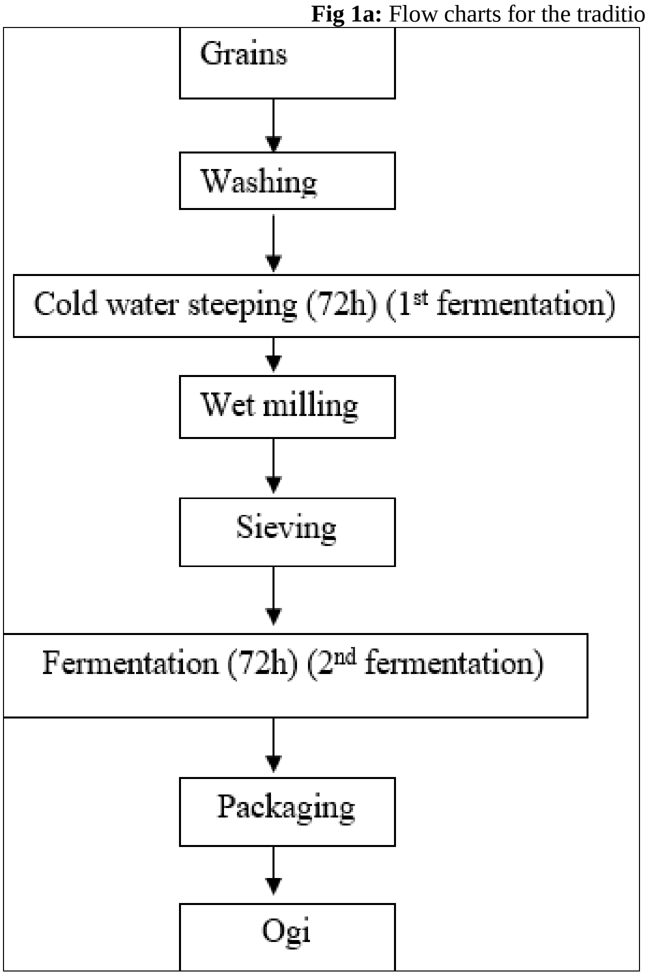
Sample Collection

A total of two hundred last stage effluents (consisting of 100 each from Ogi and cassava during processing), were randomly collected in a 10 ml wide mouthed sterile sample container from processing homes in Ezza-North L.G.A for 20 days. All the samples were collected at the last stage of processing which is the stage in which the effluents where been drained out of the Ogi and cassava Fufu respectively. The samples were conveyed in ice-cubed box to the Applied Microbiology Laboratory Unit of Ebonyi State University for analysis.



Source: Ayogu *et al*., 2014

Fig 1a: Flow charts for the traditional production of fufu from cassava Fufu



Source: Oladipupo *et al*., 2018 [25]

Fig 1b: Flow charts for the traditional production of Ogi from grains.

Isolation, enumeration and Identification of Lactic acid Bacteria from last stage Effluents Generated during Ogi, and Cassava fufu Production

For isolation of Lactic acid bacteria, 1ml each of the two hundred (200) effluent samples collected during the last stage of ogi and fufu production from were added to 9ml of distilled water and mixed properly for 5 minutes to obtain stock solutions. 1ml of each stock solution was subjected to ten folds serial dilution. Then 0.1ml of dilution factor four sample mixtures from each was pour plated out on De Man Rogosa sharp agar which was prepared and sterilized according to the manufacturer's instructions. 0.1% w/v of nystatin was added to the medium to inhibit fungal growth. The plates were incubated anaerobically using an anaerobic jar (Gas pak) with CO_2 generating kit at 30 °C for 2 days.

After the appearance of visible growth on the plates, well-isolated colonies with distinct cultural differences such as different shapes, size or colour were counted using colony counter and then purified through repeated rounds of subculturing on fresh MRS agar until pure colonies were obtained. These were stored on MRS agar slopes in the refrigerator at 4 °C. The pure colonies obtained were identified by morphological and biochemical characterization according to Cheesbrough (2006). Further confirmation of pure culture strain were subsequently identified via Vitex 2 compact 60 next-generation automated system (BIOMERIEUX, France).

Results

Lactic Acid Bacterial Count of last stage Effluents Generated during Ogi, and Cassava Fufu Production

The lactic acid bacteria count, of effluents generated during ogi, and cassava fufu production are shown in table 1. It revealed that the lactic acid bacteria count ranges from 7.0×10^4(Cassava Fufu effluent collected from Nkomoro community with isolate code 173) to 8.7×10^5 (Ogi effluent from Okposi Umuoghara community with isolate code 25). The high LAB counts are proof that LAB are one of the utmost fermenting microbiota of the effluents of ogi and cassava fufu.

Table 1: Lactic Acid Bacterial Count of Samples from last stage Effluents Generated during Ogi, and Cassava Fufu Production

S. N.	Locations	Sources/ Effluents	Range of Lactic acid Bacteria Count (log cfu/ml)
1	Ndieguazu	Ogi	7.9×10^4 - 8.7×10^5
		Cassava Fufu	6.1×10^5 - 8.0×10^5
2	Okposi	Ogi	7.7×10^5 - 8.8×10^5
		Cassava Fufu	9.2×10^4 - 8.0×10^5
3	Oriuzor	Ogi	7.6×10^5 - 8.5×10^5
		Cassava Fufu	9.7×10^4 - 8.2×10^5
4	Umuezeokoha	Ogi	7.7×10^5 - 8.6×10^5
		Cassava Fufu	8.9 x 10^4 - 8.2×10^5
5	Umuezeoka	Ogi	7.7×10^5 - 8.4×10^5
		Cassava Fufu	8.3×10^4 - 8.1×10^5
6	Ekka	Ogi	7.8×10^5 - 8.6×10^5
		Cassava Fufu	9.6×10^4 - 8.3×10^5
7	Iyere	Ogi	7.7×10^5 - 8.7×10^5
		Cassava Fufu	7.9×10^4 - 8.3×10^5
8	Ogboji	Ogi	7.6×10^5 - 8.4×10^5
		Cassava Fufu	7.7×10^4 - 8.3×10^5
9	Nkomoro	Ogi	7.6×10^5 - 8.3×10^5
		Cassava Fufu	7.0×10^4 - 7.7×10^5
10	Amuda/Amawula	Ogi	7.7×10^5 - 8.6×10^5
		Cassava Fufu	7.1×10^4 - 8.1×10^5

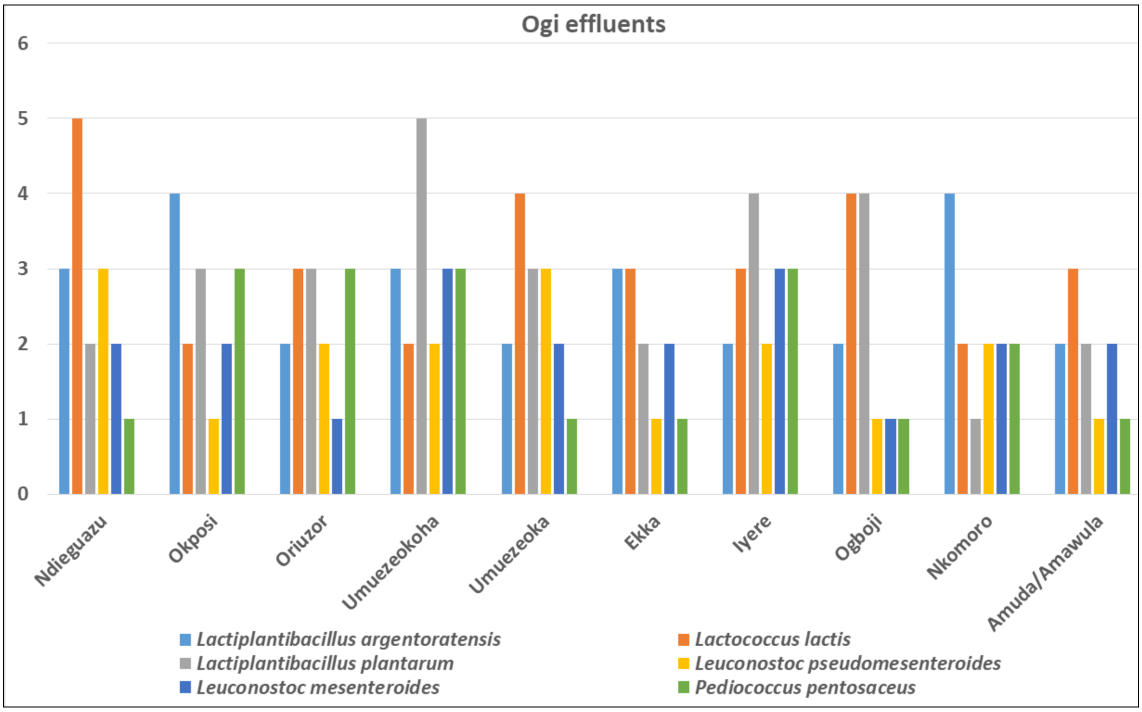


Fig 2: Distribution of Lactic Acid Bacteria from last stage Effluents Generated during Ogi Production

The frequency of LAB isolates from ogi last stage effluents according to different locations were as follows

Ogi effluents accounted for 113 LAB isolates comprising of *Lactiplantibacillus argentoratensis* (n=20), *Lactococcus lactis* (n=24), *Lactiplantibacillus plantarum* (n=24), *Leuconostoc pseudomesenteroides* (n=14), *Leuconostoc mesenteroides* (*n*=16) *Pediococcus pentosaceus* (*n*= 15). The frequency of LAB according to location sampled effluent were as follows:

- **Ndieguazu:** *Lactiplantibacillus argentoratensis* (n=3), *Lactococcus lactis*(n=5), *Lactiplantibacillus plantarum*(n=2), *Leuconostoc pseudomesenteroides* (n=3), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*= 1).
- **Okposi:** *Lactiplantibacillus argentoratensis* (n=4), *Lactococcuslactis* (n=3), *Lactiplantibacillusplantarum* (n=2), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*=3)
- **Oriuzor:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcus lactis* (n=3), *Lactiplantibacillus plantarum* (n=3), *Leuconostoc pseudomesenteroides* (n=2), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (*n*= 3).
- **Umuezeokoha:** *Lactiplantibacillus argentoratensis* (n=3), *Lactococcus lactis* (n=2), *Lactiplantibacillus plantarum* (n=5), *Leuconostoc pseudomesenteroides* (n=2), *Leuconostoc mesenteroides* (*n*=3), *Pediococcus pentosaceus* (*n*= 3)
- **Umuezeoka:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcus lactis* (n=4), *Lactiplantibacillus plantarum* (n=3), *Leuconostoc pseudomesenteroides* (n=3), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*=1)
- **Ekka:** *Lactiplantibacillus argentoratensis* (n=3), *Lactococcuslactis* (n=3), *Lactiplantibacillus plantarum* (n=2), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*= 1)
- **Iyere:** *Lactiplantibacillus argentoratensis*(n=2), *Lactococcuslactis* (n=3), *Lactiplantibacillus plantarum* (n=4), *Leuconostoc pseudomesenteroides* (n=2), *Leuconostoc mesenteroides* (*n*=3), *Pediococcus pentosaceus* (*n*=3)
- **Ogboji:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcuslactis*(n=4), *Lactiplantibacillus plantarum* (n=4), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (*n*=1)
- **Nkomoro:** *Lactiplantibacillus argentoratensis* (n=4), *Lactococcuslactis* (n=2), *Lactiplantibacillus plantarum* (n=1), *Leuconostoc pseudomesenteroides* (n=2), *Leuconosto cmesenteroides* (*n*=2), *Pediococcuspentosaceus*(*n*=2)
- **Amuda/Amawula:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcus lactis* (n=3), *Lactiplantibacillus plantarum* (n=2), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*= 1)

The frequency of LAB isolates from Fufu last stage effluents according to different locations were as follows:

Fufu effluent accounted for 122 LAB comprising of *Lactiplantibacillus argentoratensis* (n=25), *Lactococcus lactis* (n=26), *Lactiplantibacillus plantarum* (n=19), *Leuconostoc pseudomesenteroides* (n=20), *Leuconostoc mesenteroides* (*n*=15) *Pediococcus pentosaceus* (*n*=17). The frequency of LAB according to location of sampled effluents.

- **Ndieguazu:** *Lactiplantibacillus argentoratensis* (n=1), *Lactococcus lactis* (n=3), *Lactiplantibacillus plantarum* (n=1), *Leuconostoc pseudomesenteroides* (n=3), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (*n*= 1).

- **Okposi:** *Lactiplantibacillu sargentoratensis* (n=2), *Lactococcus lactis* (n=3), *Lactiplantibacillus plantarum* (n=1), *Leuconostoc pseudomesenteroides* (n=2), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (*n*=3).

- **Oriuzor:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcus lactis* (n=4), *Lactiplantibacillus plantarum* (n=2), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*=2).

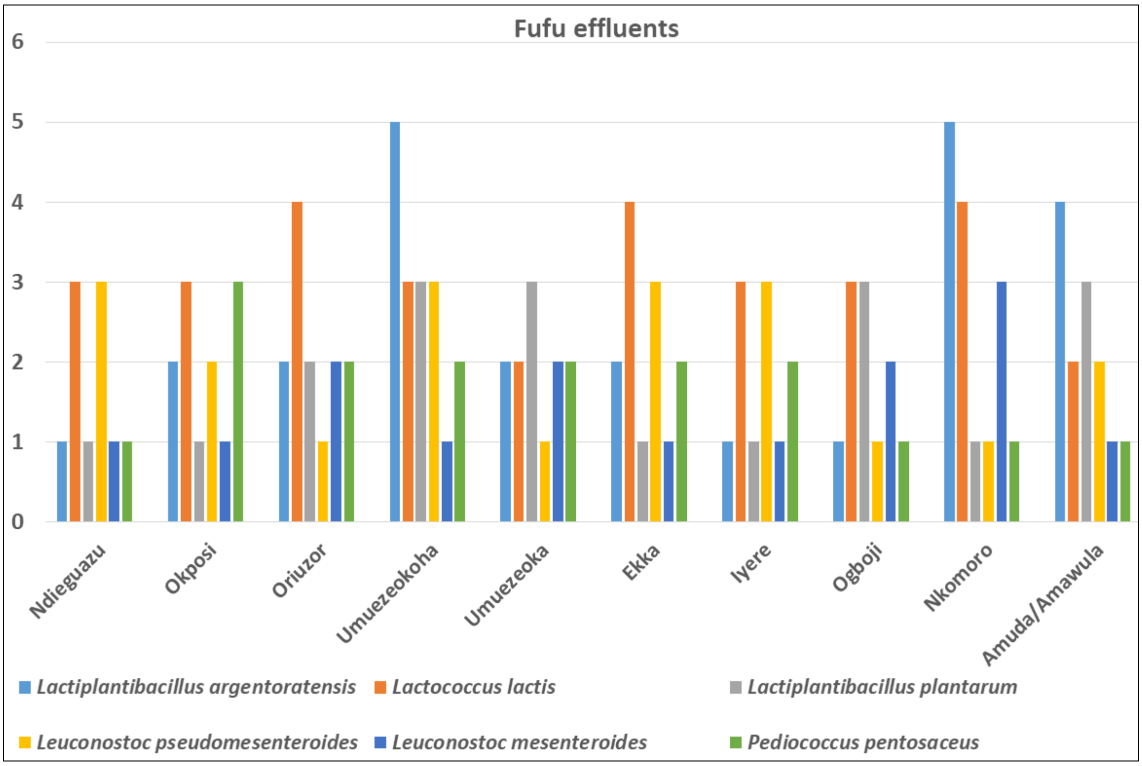


Fig 3: Distribution of Lactic Acid Bacteria from last stage Effluents Generated during Fufu Production

- **Umuezeokoha:** *Lactiplantibacillus argentoratensis* (n=5), *Lactococcuslactis* (n=3), *Lactiplantibacillus plantarum* (n=3), *Leuconostoc pseudomesenteroides* (n=3), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (n=2).
- **Umuezeoka:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcus lactis* (n=2), *Lactiplantibacillus plantarum* (n=3), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*=2).
- **Ekka:** *Lactiplantibacillus argentoratensis* (n=2), *Lactococcus lactis* (n=4), *Lactiplantibacillus plantarumn* (n=1), *Leuconostoc pseudomesenteroides* (n=3), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (*n*=2)
- **Iyere:** *Lactiplantibacillus argentoratensis* (n=1), *Lactococcus lactis* (n=3), *Lactiplantibacillus plantarum* (n=1), *Leuconostoc pseudomesenteroides* (n=3), *Leuconostoc mesenteroides* (*n*=1), *Pediococcus pentosaceus* (*n*=2)
- **Ogboji:** *Lactiplantibacillus argentoratensis*(n=1), *Lactococcus lactis* (n=3), *Lactiplantibacillus plantarum* (n=3), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=2), *Pediococcus pentosaceus* (*n*= 1)
- **Nkomoro:** *Lactiplantibacillus argentoratensis* (n=5), *Lactococcus lactis* (n=4), *Lactiplantibacillus plantarum* (n=1), *Leuconostoc pseudomesenteroides* (n=1), *Leuconostoc mesenteroides* (*n*=3), *Pediococcus pentosaceus* (*n*=1)

- **Amuda/Amawula:** *Lactiplantibacillus argentoratensis* (n=4), *Lactococcus lactis* (n=2), *Lactiplantibacillus plantarum* (n=3), *Leuconostoc pseudomesenteroides* (n=2), *Leuconostoc mesenteroides* (n=1), *Pediococcus pentosaceus* (*n*= 1).

Discussion

The result of LAB counts of the Cassava fufu and Ogi effluents in this study is not similar to the work of Uzoh *et al*. 2022 that reported that the LAB counts from locally fermented food condiment-Ogiri ranges from 2.0×10^{2} CFU/ml to 2.0×10^{4} CFU/ml while Banwo *et al.* (2023) found out at the end of the 96 h fermentation period, the LAB counts in millet, sorghum, white maize, and yellow maize ogi had reached 1.56×10^{11}, 1.46×10^{11},1.12×10^{11}, and 1.21×10^{11}cfu mL^{-1} respectively. The high lactic acid bacterial counts noted in this study depends on the optimum pH (3-5-9.6), temperature (5-45 °C), nutrients presence in the effluents (such as amino acids, peptitdes, nucleotides and vitamins), osmotic pressure necessary for growth and reproduction of LAB. Moreover, the presence of water is necessary condition for LAB reproduction.

The colonies that grew on the MRS agar media were phenotypically identified by checking the colony morphology (shape, color on MRS agar, Vitex 2 compact). Lactic acid bacteria isolated from the effluents generated from Ogi and Cassava fufu production are *Lactococcus lactis*, *Lactiplantibacillus plantarum*, and *Leuconostoc mesenteroides*. These LAB were common strains reported by numerous researchers (Ayodeji *et al.*, 2017; Olatunde *et al.*, 2018; Ohaegbu *et al.*, 2022; Kayitesi *et al.*, 2023;

Banwo *et al.,* 2023) [22, 21, 24, 10, 11]. Additionally, several studies reported the isolation and identification of similar LAB from effluents from ogi, fufu and garri production (Oladipupo *et al*., 2018, Agwaranze *et al.,* 2018 and Atta *et al.,* 2020) [25]. The results correlate with the reports of Busayo *et al*. (2017) and Oladipupo *et al*. (2018) that reports the isolation of LAB from effluents generated during cassava and ogi production respectively. Also this is similar to the works of Ohaegbu *et al.* (2022) [24] that reported the isolation of 11 LAB isolates from ogi and fufu while Wassie and Wassie, 2016 reported the isolation of 85 LAB isolates from raw cow milk in Ethiopia.

Contrary to most reported study in Nigeria on ogi and fufu effluent, it has to be highlighted, however, that our study is the first to report the isolation of *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus*. *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus* strains were found in the effluents, and their existence may be linked to the presence of many distinct substances (Endogenous microflora) in the fermentation mix. It is unclear what mechanism (s) these compound may use to promote the growth of *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus*. One theory is that these compounds serve as an additional source of energy for the bacteria. But these species have been only reported in Poland (Sionek *et al.,* 2023; Sionek *et al.,* 2024) [5, 6]. In addition, the viability of the *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus* in other study perhaps affected by many biological factors, such as the type of strain, bacterial growth interactions with starter cultures and the natural microbiota of the product, sensitivity to metabolites produced by other competing bacteria, and various pathogenic and putrefactive microorganisms (Hossain *et al.,* 2017; Terpou *et al.,* 2019) [4, 3].

It is worth noting that, LAB play an important role in shaping the sensory quality of fufu and Ogi: odor, flavor, color, and texture. Our results indicate that there is a significant presence of various LAB strains in fufu and Ogi last stage effluents. This provides a foundation for further research on additional LAB purification steps for probiotic application. Additionally, evaluation of the *in vivo* probiotic properties of these potential *Lactobacillus* strains is recommended.

Conclusion

Our results revealed that Ogi and fufu processing last stage effluents are suitable sources of LAB. Utilizing the last stage effluents from Ogi and fufu processing resulted in isolating *Leuconostoc pseudomesenteroides* and *Pediococcus pentosacesus* as new strains in Nigeria as earlier reported in Poland. Thus, the probiotic property of the LAB recovered in our investigation has to be further evaluated.

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