MALDI-TOS MS IDENTIFICATION AND ANTIBACTERIAL ACTIVITY OF LACTIC ACID BACTERIA AND YEASTS FROM OGI

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Abstract. Ogi is a traditional fermented cereal gruel widely consumed in Nigeria and other West African countries. Its fermentation involves diverse microorganisms, particularly lactic acid bacteria (LAB) and yeasts, which contribute to the characteristics of the final product. This study identified LAB and yeasts isolated from ogi using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) and evaluated the antibacterial activity of the LAB isolates. Ten ogi samples obtained from different markets within Ibadan metropolis, Oyo State, Nigeria, were subjected to microbiological isolation using MRS and potato dextrose agars. Purified LAB and yeast isolates were identified using MALDI-TOF MS, while cell-free supernatants of selected LAB isolates were evaluated against Salmonella typhimurium, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa using agar disc diffusion. Lactiplantibacillus plantarum was the predominant LAB, accounting for 59% of isolates, followed by Limosilactobacillus fermentum (15%) and Levilactobacillus brevis (9.9%). Among the yeasts, Issatchenkia orientalis showed the highest occurrence (34.9%), followed by Pichia occidentalis (32.6%) and Geotrichum candidum (27.9%). Antibacterial activity varied among LAB isolates. L. plantarum OG58 produced the highest inhibition against E. coli (10.8 mm) and showed considerable activity against S. typhimurium (9.4 mm), whereas none of the tested isolates inhibited B. subtilis. The findings demonstrate the microbial diversity of ogi and the effectiveness of MALDI-TOF MS for identifying associated LAB and yeasts. Selected LAB isolates exhibited measurable antibacterial properties, indicating their potential for further investigation in controlled food fermentation and related applications.
Keywords: *antibacterial activity, lactic acid bacteria, MALDI-TOF MS, Ogi*

Introduction

Maize (Zea mays) is employed in the production of staple food in different parts of the world and represents an important cereal crop for the preparation of several traditional fermented foods (Okeke et al., 2015). In Nigeria and some other West African countries, it is traditionally employed in the production of ogi by submerged fermentation. Ogi is a complementary weaning food for infants and young children, convenient food for the sick, convalescent and elderly or fast breakfast mostly for the rural dwellers who are low-income earners (Steinkraus, 1996). The preparation and consumption of ogi have therefore remained important aspects of traditional food processing and nutrition in many communities. Food fermentation is an old technology which is being carried out by most communities in tropical Africa (Ijabadeniyi, 2007). The origin of fermentation is difficult to trace in West Africa and is under documented as a result of lack of writing culture (Badmos et al., 2014). Traditional fermentation generally involves the activities of naturally occurring microorganisms that transform the physicochemical, sensory and microbiological characteristics of food materials

during processing. Eze et al. (2014) reported that traditional food fermentation is a biotechnological process which makes use of microorganisms found in fresh food products in an economical and empirical method for food preservation which enhances its organoleptic and nutritional quality. The traditional production of ogi involves different processing operations, including steeping and fermentation, during which complex microbial populations may develop and contribute to the characteristics of the final product (Omemu et al., 2018; Akinleye et al., 2014; Ojokoh, 2009; Teniola et al., 2005; Nago et al., 1998). Consequently, understanding the microorganisms involved in ogi fermentation is important not only for describing the traditional fermentation process but also for improving the microbiological quality, consistency, safety and desirable characteristics of this widely consumed fermented cereal product.

The microorganisms typically associated with fermentation of ogi include lactic acid bacteria (LAB) and yeasts, which constitute important microbial groups responsible for many of the biochemical transformations that occur during fermentation. These microorganisms play important roles in aroma development, microbial stability and flavor enhancement during the production of fermented cereal products (Omemu, 2011). Studies of ogi fermentation have demonstrated that the microbial population changes considerably during fermentation and that LAB and yeasts are important components of the fermenting microbial community (Omemu et al., 2018; Izah et al., 2016; Akinleye et al., 2014; Ijabadeniyi, 2007; Nago et al., 1998). Omemu et al. (2018), for instance, assessed the microbiological characteristics of maize ogi cofermented with pigeon pea and reported the occurrence of several LAB and yeast species during fermentation. The study demonstrated changes in acidity and microbial populations during fermentation and identified LAB such as Lactobacillus buchneri, Lactobacillus casei, Lactobacillus pentosus and Pediococcus pentosaceus together with different yeast species. Such observations support the important role of microbial interactions in determining the properties of fermented ogi. The microbiological quality of fermented maize ogi is particularly important because traditional processing conditions may permit different microorganisms to participate in fermentation (Izah et al., 2016). Previous investigations have therefore examined microbial diversity, fermentation dynamics and microbiological changes associated with ogi production using conventional microbiological, phenotypic and molecular approaches (Omemu et al., 2018; Okeke et al., 2015; Omemu, 2011; Ijabadeniyi, 2007; Nago et al., 1998). In addition, Adesokan (2019) investigated the characterization and functional properties of yeasts associated with traditional fermented beverages and cereal gruel. Collectively, these studies demonstrate that accurate characterization of LAB and yeasts is important for understanding the fermentation process and identifying microorganisms that may possess desirable technological and functional characteristics.

Despite the established importance of LAB and yeasts in fermented foods, accurate and rapid identification of these microorganisms remains an important consideration in microbiological studies of traditional fermented products. Previous identification of LAB and yeast isolates from ogi has relied substantially on phenotypic, biochemical and molecular approaches (Adesokan, 2019; Omemu et al., 2018; Okeke et al., 2015; Nago et al., 1998). Although these approaches are valuable for microbial characterization, there is a need for rapid identification techniques capable of differentiating microorganisms through reproducible microbial profiles. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) provides an alternative approach based on the analysis of microbial protein profiles. Analysis of

protein profiles of microorganisms carried out using MALDI-TOF MS has been applied to the identification of lactic acid bacteria, demonstrating its potential as a rapid method for microbial identification (Karaduman et al., 2017; Nacef et al., 2016). Karaduman et al. (2017), for example, applied MALDI-TOF mass spectrometry for identification of LAB isolated from non-commercial yogurts, while Nacef et al. (2016) applied the method to LAB isolated from French cheese. More recently, MALDI-TOF MS profiling has also been applied to LAB obtained from a Nigerian cereal-based fermented beverage, demonstrating the relevance of this technique to indigenous fermented cereal products (Ogunremi et al., 2022). However, the available literature in the supplied references indicates comparatively limited application of MALDI-TOF MS specifically to the simultaneous identification of LAB and yeasts isolated from ogi. Furthermore, identification alone does not establish whether individual LAB isolates possess potentially useful antibacterial properties. This creates a need to combine rapid microbial identification with functional assessment of the isolates, particularly because antibacterial activity may provide additional information concerning their potential relevance in fermented-food microbial ecology and possible technological applications.

Therefore, the present study focuses on the identification of lactic acid bacteria and yeasts isolated from ogi using MALDI-TOF MS and the subsequent determination of the antibacterial activity of the LAB isolates. The application of MALDI-TOF MS is intended to provide protein-profile-based identification of the microorganisms associated with the fermented cereal product, complementing conventional approaches that have previously been employed for the characterization of microorganisms associated with ogi and other fermented foods (Adesokan, 2019; Omemu et al., 2018; Karaduman et al., 2017; Nacef et al., 2016; Okeke et al., 2015). Determination of antibacterial activity is also relevant because LAB isolated from fermented products may exhibit inhibitory activity against other microorganisms. Sanam et al. (2022), for example, investigated antibacterial activity of LAB against Bacillus cereus, Staphylococcus aureus and Escherichia coli, demonstrating the usefulness of evaluating LAB beyond taxonomic identification. Similarly, characterization of LAB from fermented cereal beverages using MALDI-TOF MS provides an opportunity to connect microbial identity with potentially useful functional properties (Ogunremi et al., 2022). Accordingly, the specific objectives of this study are to isolate lactic acid bacteria and yeasts associated with ogi, identify the recovered LAB and yeast isolates using MALDI-TOF MS, and determine the antibacterial activity of the identified LAB isolates. By integrating microbial identification with antibacterial assessment, the study is expected to provide additional information on the diversity and functional potential of microorganisms involved in ogi fermentation. Such information may contribute to a better understanding of the microbiology of traditional ogi fermentation and provide a basis for subsequent investigations into potentially beneficial LAB isolates for controlled fermentation, microbial quality improvement and other food-related applications.

Materials and Methods

Sample collection

Traditional ogi samples were collected for the isolation and subsequent characterization of lactic acid bacteria (LAB) and yeasts associated with the fermented cereal product. The isolation of these microorganisms is important because the

fermentation of ogi involves a diverse microbial population, particularly LAB and yeasts, which contribute to the biochemical and microbiological changes that occur during fermentation. Previous investigations of traditionally fermented ogi have demonstrated the presence of different microorganisms and considerable changes in microbial populations throughout the fermentation process (Omemu et al., 2018; Izah et al., 2016; Okeke et al., 2015; Akinleye et al., 2014; Omemu, 2011; Ijabadeniyi, 2007; Nago et al., 1998). These microorganisms are associated with the fermentation characteristics of the cereal substrate and may influence the quality and properties of the final product. Since traditionally produced ogi is commonly prepared under conditions in which fermentation depends largely on naturally occurring microorganisms, samples obtained from different locations may contain diverse populations of LAB and yeasts. Consequently, the collection of ogi from different markets was intended to increase the possibility of recovering representative microbial isolates from traditionally prepared products available to consumers. This approach was also relevant to the main objective of the present study, which was to obtain LAB and yeast isolates from ogi for subsequent identification using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) and to further investigate the antibacterial activity of the recovered LAB isolates. The isolation stage therefore provided the microbial cultures required for both the identification and functional assessment components of the study.

About 10 different samples of ogi were purchased from different markets within Ibadan metropolis, Oyo State, Nigeria, and transported to the laboratory for isolation of LAB strains and yeasts. The samples were processed microbiologically using serial dilution and culture-based isolation procedures. Ten-fold serial dilutions of each ogi sample were prepared using sterile distilled water to reduce the microbial concentration and facilitate the recovery of discrete colonies on appropriate microbiological media. Appropriate dilutions were pour-plated onto de Man, Rogosa and Sharpe (MRS) agar for the isolation of LAB strains and potato dextrose agar (PDA) for the isolation of yeasts. The use of culture media for recovering microorganisms associated with ogi is consistent with previous microbiological investigations of fermented cereal products in which LAB and yeasts were isolated and subsequently characterized (Omemu et al., 2018; Izah et al., 2016; Akinleye et al., 2014; Omemu, 2011; Ijabadeniyi, 2007). Following microbial growth, distinct colonies obtained on the respective culture media were selected for purification. The microbial isolates were purified by repeated streaking on sterile culture media until pure cultures were obtained. Purification was necessary to ensure that individual cultures rather than mixed microbial populations were subjected to subsequent MALDI-TOF MS identification. The purified LAB and yeast isolates obtained from this procedure constituted the working cultures used for further analysis. Fresh cultures of the isolates were subsequently prepared for protein extraction and MALDI-TOF MS analysis, while the LAB isolates were additionally investigated for their ability to inhibit selected spoilage and pathogenic microorganisms.

Identification by MALDI TOF MS

Accurate identification of microorganisms isolated from fermented foods is important for understanding the microbial diversity associated with fermentation and for relating individual microorganisms to their possible functional properties. Conventional phenotypic and biochemical identification approaches may be supplemented by techniques that characterize microorganisms based on their molecular or protein

profiles. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been applied for the identification and profiling of LAB associated with fermented foods. Nacef et al. (2016) investigated the use of MALDI-TOF mass spectrometry for the identification of LAB isolated from French cheese, while Karaduman et al. (2017) used MALDI-TOF MS for the identification of LAB recovered from non-commercial yogurts. More recently, Ogunremi et al. (2022) applied MALDI-TOF MS profiling to LAB isolated from kunu-zaki, a cereal-based Nigerian fermented beverage. These studies demonstrate the applicability of MALDI-TOF MS to microorganisms associated with fermented food matrices and provide methodological support for its application to isolates obtained from ogi. In the present study, MALDI-TOF MS was therefore employed to identify the purified LAB and yeast isolates recovered from the ogi samples. The objective of this stage was to obtain reliable identification of the microbial isolates based on their protein profiles and the corresponding identification scores generated by the MALDI BioTyper system. The application of MALDI-TOF MS was particularly relevant because the study sought not only to isolate microorganisms associated with ogi but also to establish their identities before evaluating the functional antibacterial properties of the LAB isolates.

Fresh cultures of the purified LAB and yeast isolates were obtained and subjected to protein extraction and treatment for MALDI-TOF MS analysis as previously described by Ogunremi et al. (2022). Measurements were carried out using a Reflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany), while the resulting data were analyzed using Bruker Daltonics MALDI BioTyper Software. The protein mass spectra generated for individual microbial isolates were compared using the software to obtain identification scores corresponding to the degree of similarity with reference profiles. Interpretation of the MALDI BioTyper log scores was performed according to the identification ranges used in this study. Log scores ranging from 2.300 to 3.000 were interpreted as highly probable species-level identification, while scores ranging from 2.000 to 2.299 indicated highly probable genus-level identification and probable species-level identification. Scores between 1.700 and 1.999 were considered indicative of probable genus-level identification, whereas scores from 0.000 to 1.699 were regarded as providing no reliable identification. These score categories were used to determine the confidence associated with the identification of each LAB and yeast isolate obtained from the ogi samples. The resulting microbial identities provided the basis for describing the LAB and yeast populations recovered in the study. In addition, the identification of the LAB isolates before antibacterial testing made it possible to relate any observed inhibitory activity to the identified isolates rather than to unidentified microbial cultures.

Antibacterial activity of the LAB isolates

The antibacterial activity of the LAB isolates obtained from ogi was investigated to determine whether metabolites present in their cell-free supernatants could inhibit selected spoilage and pathogenic microorganisms. In addition to their contribution to fermentation, LAB from fermented foods may exhibit functional properties that can be assessed through antibacterial testing. Sanam et al. (2022), for example, investigated the antibacterial activity of LAB isolated from Sumba mare's milk against Bacillus cereus, Staphylococcus aureus and Escherichia coli. Such investigations demonstrate the relevance of examining LAB not only according to their taxonomic identities but also according to their potential inhibitory properties. The present antibacterial assessment

was therefore designed as the functional component of the study following isolation and MALDI-TOF MS identification. The antimicrobial activity of LAB isolates from ogi was assessed against common spoilage and pathogenic microorganisms, namely Salmonella typhimurium, Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. These test microorganisms were grown on Mueller-Hinton agar prior to antibacterial assessment. The objective was to determine whether cell-free products generated by the LAB isolates were capable of producing measurable inhibition against the selected microorganisms. Evaluation of this property provided additional information beyond microbial identification and enabled the functional potential of the LAB isolates recovered from ogi to be investigated.

Cell-free supernatants of the LAB isolates were prepared using fresh cultures of the LAB isolates grown in sterile MRS broth. Following incubation of the cultures, 1,200 µL aliquots of cultures at the stationary phase were aseptically transferred into sterile Eppendorf tubes. The aliquots were centrifuged at 10,000 × g for 12 min to separate bacterial cells and other particulate materials from the liquid culture fraction. Following centrifugation, the resulting supernatants were carefully collected without disturbing the deposited cellular material. The recovered supernatants were subsequently filter-sterilized using sterile 0.2 µm nylon syringe filters to produce cell-free LAB supernatants (CFS). Filtration was performed to remove residual bacterial cells from the supernatants so that the antibacterial assessment was based on the activity associated with the cell-free fraction rather than the direct growth or competitive activity of viable LAB cells. The resulting CFS preparations were subsequently used in the agar disc diffusion assay against S. typhimurium, B. subtilis, E. coli, S. aureus and P. aeruginosa. This procedure enabled the antibacterial activity associated with each LAB isolate to be evaluated through its corresponding cell-free supernatant. The production of the CFS therefore represented an important intermediate stage between cultivation of the identified LAB isolates and determination of their inhibitory effects using the agar disc diffusion procedure.

The agar disc diffusion assay (ADDA)

The agar disc diffusion assay (ADDA) was employed to assess the antibacterial effects of the cell-free supernatants produced by the LAB isolates against the selected spoilage and pathogenic microorganisms. Disc diffusion provides a means of observing inhibitory activity through the formation of a clear zone surrounding a disc containing the test preparation after incubation on an agar surface inoculated with the target microorganism. In the present study, the assay was used to determine whether extracellular products contained in the CFS of the ogi-derived LAB isolates could inhibit Salmonella typhimurium, Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The inclusion of different test organisms enabled the inhibitory activity of the LAB isolates to be examined against several bacterial targets rather than against a single microorganism. Assessment of antibacterial activity is relevant to the functional characterization of LAB, as demonstrated by previous investigations in which LAB isolated from fermented products were examined against bacterial organisms such as B. cereus, S. aureus and E. coli (Sanam et al., 2022). In the present investigation, antibacterial testing followed MALDI-TOF MS identification so that the inhibition observed during the assay could subsequently be associated with identified LAB isolates. The purpose of the ADDA was therefore to provide measurable

evidence of antibacterial activity based on the zones of inhibition generated around discs exposed to the cell-free supernatants.

BD Sensi-Discs were aseptically immersed in the sterile CFS preparations for 5 min to allow absorption of the supernatant. Following exposure to the CFS, the discs were aseptically transferred onto the surface of Mueller-Hinton agar plates previously seeded with lawns of the selected spoilage and pathogenic microorganisms, namely S. typhimurium, B. subtilis, E. coli, S. aureus and P. aeruginosa. Care was taken during disc placement to maintain aseptic conditions and prevent contamination of the assay plates. After placement of the CFS-treated discs, the plates were left at room temperature for 4 h to allow components of the cell-free supernatants to diffuse from the discs into the surrounding Mueller-Hinton agar. Thereafter, the plates were incubated overnight at 30 °C to permit growth of the test microorganisms and development of observable inhibition zones around discs exhibiting antibacterial activity. Following incubation, the plates were examined for clear zones surrounding the discs. The diameters of the zones of inhibition were measured using an electronic digital caliper (Powerfix®). The measured inhibition-zone diameters were used as indicators of the antibacterial activity exhibited by the respective LAB cell-free supernatants against each test microorganism. Larger measurable zones represented greater inhibition under the conditions employed in the assay, whereas the absence of a measurable inhibition zone indicated that no detectable antibacterial effect was observed under the experimental conditions.

Results and Discussion

The identification of lactic acid bacteria (LAB) isolated from ogi using MALDI-TOF MS revealed considerable diversity among the isolates, although Lactiplantibacillus plantarum was clearly the predominant species recovered in the present study. As presented in *Table 1*, L. plantarum had the highest percentage of occurrence of 59%, representing 54 of the LAB isolates, and this was followed by Limosilactobacillus fermentum with 15% and Levilactobacillus brevis with 9.9%. Lactiplantibacillus pentosus accounted for 6.6% of the isolates. In contrast, Enterococcus faecalis, Companilactobacillus paraplantarum, Lactiplantibacillus paraplantarum, Limosilactobacillus ingluviei, Companilactobacillus formosensis, Companilactobacillus mishanensis and Lactococcus allomyrinae had the least percentage of occurrence of 1.1% each. The predominance of lactobacilli observed in the present study is consistent with previous reports on the microbiology of ogi fermentation. Lactic acid bacteria have been reported as important microorganisms associated with the fermentation of maize into ogi, although the species composition may vary according to fermentation conditions, raw materials and identification methods. In another study, lactic acid bacteria were isolated from ogi cofermented with pigeon pea, and the isolates reported included Lactobacillus buchneri, L. casei, L. pentosus and Pediococcus pentosaceus (Omemu et al., 2018). Similarly, Lactobacillus plantarum and L. fermentum, among others, were isolated from ogi when phenotypic methods of identification were employed (Akinleye et al., 2014). Lactobacilli have also been reported in the fermentation of maize to ogi in the Benin Republic (Nago et al., 1998). Furthermore, LAB such as Lactococcus raffinolactis, Pediococcus sp., Pediococcus pentosaceus, Lactobacillus plantarum, L. suebicus and L. brevis were isolated during batch fermentation of ogi using back-slopping (Teniola et al., 2005). Izah et al. (2016)

similarly reported Lactobacillus species among the predominant microorganisms associated with maize ogi. Okeke et al. (2015) reported that lactic acid bacteria constituted a considerable proportion of bacteria recovered during maize fermentation. The high occurrence of L. plantarum observed in the present study therefore supports earlier findings indicating that members of the LAB group, particularly lactobacilli, constitute an important component of the microbial population associated with ogi fermentation.

Table 1. *Frequency of occurrence of lactic acid bacteria isolated from ogi-a West African cereal gruel.*

SN	Name of LAB isolates	Number of isolates	Percentage of isolates
1	Lactiplantibacillus pentosus	6	6.6
2	Lactiplantibacillus plantarum	54	59
3	Limosilactobacillus fermentum	14	15
4	Levilactobacillus brevis	9	9.9
5	Enterococuss faecalis	1	1.1
6	Companilactobacillus paraplantarum	1	1.1
7	Lactiplantibacillus paraplantarum	1	1.1
8	Limosilactobacillus ingluviei	1	1.1
9	Companilactobacillus formosensi	1	1.1
10	Companilactobacillus mishanensis	1	1.1
11	Lactococcus allomyrinae	1	1.1

The frequency of occurrence of yeasts isolated from ogi is presented in *Table 2*. Five yeast species were identified, with Issatchenkia orientalis having the highest percentage of occurrence of 34.9%, corresponding to 15 isolates. This was closely followed by Pichia occidentalis, which accounted for 32.6% with 14 isolates, while Geotrichum candidum represented 27.9% with 12 isolates. Candida rugosa and Candida tropicalis had the least percentage of occurrence, with each accounting for 2.3% of the yeast isolates. The findings demonstrate that the yeast population of the ogi samples was dominated by I. orientalis, P. occidentalis and G. candidum, whereas the two Candida species occurred at considerably lower frequencies. Yeasts represent an important component of the microbial community associated with traditional cereal fermentation and may occur together with LAB during the fermentation process. Previous investigations have similarly demonstrated the occurrence of yeasts during ogi fermentation. Omemu et al. (2018) reported different yeasts during microbiological assessment of maize ogi cofermented with pigeon pea, supporting the observation that ogi fermentation involves both bacterial and yeast populations. Adesokan (2019) also investigated the characterization and functional properties of yeasts associated with traditional fermented alcoholic beverages and cereal gruel, demonstrating the relevance of yeasts to traditional fermented products. Earlier studies of traditional ogi have also described the physicochemical and microbiological characteristics of the fermentation process (Nago et al., 1998). Furthermore, Candida species had previously been reported during a comparative investigation of traditional and improved methods of fermentation of maize into ogi (Ojokoh, 2009). Although the relative proportions and identities of yeast species may differ among investigations, the present results further demonstrate the diversity of yeasts associated with traditionally fermented ogi. Differences between studies may be associated with the source of samples, fermentation conditions, processing practices and methods used for microbial identification. The detection of several yeast species alongside the diverse LAB population also emphasizes the mixed microbial nature of traditional ogi fermentation and provides additional information

concerning microorganisms that can be identified when MALDI-TOF MS is applied to isolates recovered from this traditional fermented cereal product.

***Table 2**. Frequency of occurrence of yeast isolated from ogi-a West African cereal gruel.*

SN	Yeast isolates	Number of isolates	Percentage of isolates
1	Issatchenka orientalis	15	34.9
2	Geotrichum candidum	12	27.9
3	Pichia occidentalis	14	32.6
4	Candida rugosa	1	2.3
5	Candida tropicalis	1	2.3

The antibacterial activity of the cell-free supernatants (CFS) of the LAB isolates against the selected spoilage and pathogenic organisms is presented in *Table 3*. Considerable variation was observed in the inhibitory activities of the different LAB isolates. The CFS of Lactiplantibacillus plantarum OG58 produced the highest zone of inhibition against Escherichia coli, with a diameter of 10.8 mm, and also demonstrated substantial activity against Salmonella typhimurium, producing a 9.4 mm inhibition zone. Several other L. plantarum isolates also exhibited measurable antibacterial activity against E. coli. For example, OG113 produced an inhibition zone of 10.7 mm, while OG94, OG45 and Limosilactobacillus ingluviei OG38 produced zones of 10.5 mm against E. coli. The results therefore indicate variation in antibacterial activity even among isolates identified as belonging to the same species. In contrast, some isolates showed little or no inhibitory activity against the indicator organisms. Levilactobacillus brevis OG74 and Lactiplantibacillus plantarum OG12 did not inhibit any of the indicator organisms under the conditions employed in the assay. Similarly, Limosilactobacillus fermentum OG65 showed no detectable inhibition against the five organisms. None of the LAB isolates showed measurable inhibition against Bacillus subtilis, while inhibition against Staphylococcus aureus was uncommon; only L. plantarum OG28 showed measurable activity, with a zone of 8.3 mm. The antibacterial activity of LAB against pathogenic organisms such as E. coli, Bacillus cereus and Staphylococcus aureus has previously been reported by Sanam et al. (2022). Their findings demonstrate that LAB obtained from fermented products may exhibit antibacterial properties against selected microorganisms. The variation observed among isolates in the present study indicates that antibacterial activity cannot necessarily be predicted solely from species identity because isolates belonging to the same species displayed different inhibition profiles. The results consequently support the importance of evaluating individual LAB isolates when investigating their potential antibacterial properties.

***Table 3**. Antibacterial activity of cell-free supernatants of LAB isolates against selected indicator organisms (zone of inhibition, mm).*

SN	LAB isolates	Indicator organisms				
		Escherichia coli	Staphylococuss	Bacillus subtilis	Pseudomonas Aerigunosa	Salmonella Typhimirium
1	Lactiplantibacillus plantarumOG22	9.5	0	0	8.0	8.0
2	Lactiplantibacillus plantarumOG72	10	0	0	8.3	6.7
3	Lactiplantibacillus plantarumOG78	10	0	0	9.5	7.2
4	Levilactobacillus brevisOG74	0	0	0	0	0
5	Limosilactobacillus fermentumOG88	7.1	0	0	6.6	6.9
6	Lactiplantibacillus plantarumOG28	10	8.3	0	9.6	8.2
7	Lactiplantibacillus plantarumOG94	10.2	0	0	7.6	8.1
8	Lactiplantibacillus plantarumOG58	10.8	0	0	7.4	9.4
9	Lactiplantibacillus plantarumOG94	10.5	0	0	7.4	8.4
10	Lactiplantibacillus plantarumOG78	8.2	0	0	0	7.8
11	Limosilactobacillus fermentumOG65	0	0	0	0	0

12	Lactiplantibacillus plantarumOG74B	7.7	0	0	0	7.3
13	Lactiplantibacillus plantarumOG36	8.6	0	0	0	7.7
14	Lactiplantibacillus plantarumOG39	8.6	0	0	0	9.3
15	Lactiplantibacillus plantarumOG46	8.7	0	0	0	7.1
16	Lactiplantibacillus plantarumOG24	8.4	0	0	0	0
17	Lactiplantibacillus plantarumOG103	10.1	0	0	0	7.6
18	Limosilactobacillus ingluvieiOG38	10.5	0	0	0	8.4
19	Limosilactobacillus fermentumOG49	8.4	0	0	0	0
20	Lactiplantibacillus plantarumOG11	8.1	0	0	0	0
21	Lactiplantibacillus plantarumOG74A	9.6	0	0	0	0
22	Lactiplantibacillus plantarumOG12	0	0	0	0	0
23	Lactiplantibacillus plantarumOG113	10.7	0	0	0	7.2
24	Lactiplantibacillus plantarumOG40	8.6	0	0	0	7.4
25	Lactiplantibacillus plantarumOG45	10.5	0	0	0	0
26	Lactiplantibacillus plantarumOG44	10	0	0	0	0
27	Lactiplantibacillus plantarumOG40	6.5	0	0	0	0
28	Lactiplantibacillus plantarumOG80	7.1	0	0	0	0
29	Limosilactobacillus fermentumOG102	7.2	0	0	0	0

The predominance of Lactiplantibacillus plantarum observed in the present study provides further evidence of the importance of LAB in the microbial ecology of ogi fermentation. Although several LAB species were identified, the occurrence of L. plantarum at 59% was substantially higher than that of Limosilactobacillus fermentum (15%), Levilactobacillus brevis (9.9%) and Lactiplantibacillus pentosus (6.6%). This distribution suggests that the LAB community associated with the ogi samples was characterized by a dominant species accompanied by several less frequently occurring species. Previous studies have similarly demonstrated the importance and diversity of LAB during ogi fermentation (Omemu et al., 2018; Izah et al., 2016; Okeke et al., 2015; Akinleye et al., 2014; Omemu, 2011; Ijabadeniyi, 2007; Teniola et al., 2005; Nago et al., 1998). For example, Omemu et al. (2018) reported Lactobacillus buchneri, Lactobacillus casei, Lactobacillus pentosus and Pediococcus pentosaceus during microbiological assessment of maize ogi cofermented with pigeon pea. Akinleye et al. (2014) also reported L. plantarum and L. fermentum among microorganisms associated with ogi production. Furthermore, Teniola et al. (2005) identified different LAB during the processing of ogi, while Nago et al. (1998) demonstrated the microbiological complexity of traditional fermented maize slurry. The variations in LAB composition among the studies may be related to differences in fermentation techniques, geographical location, processing environment, raw materials and methods used for microbial identification. Traditional fermentation may allow naturally occurring microbial populations from the raw material and processing environment to participate in the fermentation process. Consequently, differences between batches and production locations can potentially contribute to variation in microbial diversity. The identification of less frequently occurring LAB species in the present investigation is therefore also noteworthy, as it demonstrates that the microbial community associated with ogi extends beyond the dominant L. plantarum. These results reinforce the importance of comprehensive microbial identification when characterizing traditional fermented foods rather than focusing exclusively on the predominant microorganisms.

The application of MALDI-TOF MS in the present study also provides an important methodological contribution to the characterization of microorganisms associated with ogi because it enabled the identification of a relatively diverse collection of LAB and yeast isolates based on their protein profiles. MALDI-TOF MS has previously been successfully applied to LAB associated with other fermented foods and beverages, supporting its usefulness for microbial identification. Ogunremi et al. (2022) employed MALDI-TOF MS profiling for LAB obtained from kunu-zaki, a cereal-based Nigerian

fermented beverage, and identified microorganisms belonging to different LAB groups. The relevance of this finding to the present investigation is particularly important because both kunu-zaki and ogi are traditional cereal-based fermented products from Nigeria. Karaduman et al. (2017) similarly employed MALDI-TOF mass spectrometry for the identification of LAB isolated from non-commercial yogurts, while Nacef et al. (2016) applied MALDI-TOF mass spectrometry to LAB isolated from Maroilles, a French cheese. Together, these investigations indicate that MALDI-TOF MS can be applied across different fermented food matrices for microbial identification (Ogunremi et al., 2022; Karaduman et al., 2017; Nacef et al., 2016). This is particularly relevant when considered alongside earlier investigations of ogi microbial diversity, where microorganisms were characterized using other microbiological and identification approaches (Omemu et al., 2018; Okeke et al., 2015; Akinleye et al., 2014; Ijabadeniyi, 2007; Nago et al., 1998). The present findings therefore complement earlier knowledge of ogi microbiology by demonstrating the application of MALDI-TOF MS to isolates recovered from the product. The identification of Issatchenkia orientalis, Pichia occidentalis, Geotrichum candidum, Candida rugosa and Candida tropicalis also indicates considerable yeast diversity in the samples. Previous studies have established that yeasts occur as part of the microbial community of ogi and related cereal fermentations (Adesokan, 2019; Omemu et al., 2018; Ojokoh, 2009; Nago et al., 1998). Thus, the present results emphasize that characterization of ogi fermentation should consider both LAB and yeast populations because both groups constitute components of the complex microbial ecosystem associated with the traditional fermented product.

The antibacterial activity demonstrated by selected LAB isolates represents another important finding of the present investigation and provides evidence of functional differences among isolates recovered from ogi. Although several isolates were identified as Lactiplantibacillus plantarum, their cell-free supernatants did not produce identical inhibition patterns against the indicator organisms. L. plantarum OG58 produced the highest inhibition against Escherichia coli at 10.8 mm and also showed a 9.4 mm inhibition zone against Salmonella typhimurium. Other isolates showed different levels of activity, while Levilactobacillus brevis OG74, Limosilactobacillus fermentum OG65 and L. plantarum OG12 did not produce detectable inhibition against any of the five indicator organisms under the experimental conditions. The absence of activity against Bacillus subtilis across the tested isolates was also notable. These findings demonstrate that identification of isolates as members of the same LAB species does not necessarily imply that they will possess identical antibacterial properties. This observation strengthens the importance of evaluating individual isolates when selecting LAB for further functional investigations. Antibacterial activity among LAB isolated from fermented products has also been demonstrated in previous research. Sanam et al. (2022) investigated LAB isolated from Sumba mare's milk and demonstrated antibacterial activity against organisms including Bacillus cereus, Staphylococcus aureus and Escherichia coli. Although the source of the LAB and experimental conditions differed from those of the present study, their results support the broader observation that selected LAB isolates can exhibit measurable inhibitory activity against bacterial targets. The microbial diversity previously reported during ogi fermentation further indicates that traditional fermented cereal products may provide a source of LAB with differing biological characteristics (Omemu et al., 2018; Izah et al., 2016; Okeke et al., 2015; Akinleye et al., 2014). Therefore, the combination of MALDI-TOF MS identification and antibacterial screening used in the present study provides information

on both the identity and functional characteristics of the isolates. The findings suggest that selected ogi-derived LAB, particularly isolates showing relatively greater inhibition, warrant further investigation to determine the factors responsible for their antibacterial activity and their potential usefulness in controlled food-fermentation applications.

Conclusion

This study demonstrated the diversity of lactic acid bacteria (LAB) and yeasts associated with ogi, a traditional West African fermented cereal gruel, and highlighted the usefulness of MALDI-TOF MS for the identification of microorganisms recovered from the fermented product. Among the LAB identified, Lactiplantibacillus plantarum was the predominant species, accounting for 59% of the isolates, followed by Limosilactobacillus fermentum at 15%, Levilactobacillus brevis at 9.9% and Lactiplantibacillus pentosus at 6.6%. Other LAB species occurred at considerably lower frequencies, demonstrating that ogi contains a diverse LAB population despite the clear predominance of L. plantarum. Considerable diversity was also observed among the yeast isolates. Issatchenkia orientalis was the most frequently identified yeast with an occurrence of 34.9%, followed by Pichia occidentalis at 32.6% and Geotrichum candidum at 27.9%, whereas Candida rugosa and Candida tropicalis each represented 2.3% of the isolates. These findings confirm that the microbial community associated with ogi comprises both predominant and less frequently occurring LAB and yeast species. The application of MALDI-TOF MS provided an effective approach for profiling the microbial isolates recovered from the samples and enabled identification of a wider range of microorganisms associated with the fermented cereal product. The findings therefore contribute to the existing understanding of the microbiological characteristics of ogi and emphasize the complexity of the microbial population involved in its fermentation. In particular, the dominance of L. plantarum observed in this study supports the importance of LAB in ogi fermentation, while the recovery of several yeast species further demonstrates that the fermentation process involves a mixed microbial community rather than a single group of microorganisms.

The study further demonstrated that selected LAB isolates obtained from ogi exhibited antibacterial activity against some of the spoilage and pathogenic microorganisms investigated, although considerable variation occurred among individual isolates. The cell-free supernatant of Lactiplantibacillus plantarum OG58 showed the highest inhibition against Escherichia coli, producing an inhibition zone of 10.8 mm, and also exhibited considerable inhibitory activity against Salmonella typhimurium, with a zone of 9.4 mm. Several other L. plantarum isolates also showed measurable inhibitory activity, particularly against E. coli and S. typhimurium. In contrast, some isolates did not demonstrate detectable antibacterial activity under the experimental conditions, while none of the tested LAB isolates inhibited Bacillus subtilis. These differences indicate that antibacterial activity varies among individual LAB isolates, including isolates belonging to the same species, and therefore emphasizes the importance of strain-level functional assessment in addition to microbial identification. Overall, the combination of MALDI-TOF MS identification and antibacterial screening provided complementary information on both the diversity and functional characteristics of microorganisms isolated from ogi. The results indicate that traditional ogi represents a source of diverse LAB and yeasts and that selected LAB

isolates possess measurable antibacterial properties that may justify further investigation. Future studies should therefore focus on detailed characterization of the most active LAB isolates, identification of the compounds responsible for their antibacterial effects, evaluation against a broader range of food spoilage and pathogenic microorganisms, and assessment of their performance under controlled fermentation conditions. Such investigations would provide stronger evidence regarding their potential application in food fermentation and contribute to improved understanding and utilization of the beneficial microbial resources associated with traditional ogi fermentation.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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