EVALUATION OF THE SAFETY ASSESSMENT OF LYSINE-PRODUCING LACTIC ACID BACTERIA AND YEAST ISOLATED FROM *OGI*, A FERMENTED SORGHUM GRUEL

BY

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SUPERVISED BY

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DECLARATION

I, ANYAOKEI NAOMI IFEOMA, hereby declare that the project work titled **EVALUATION OF THE SAFETY ASSESMENT OF LYSINE PRODUCING LACTIC ACID BACTERIA AND YEAST ISOLATED FROM *OGI* A FERMENTED SORGHUM GRUEL** is a record of an original work done by me, as a result of my research effort carried out in the Departments of Biological Sciences and Biotechnology, Caleb University Imota, Lagos.

……..… **….……..……**

SIGNATURE DATE

CERTIFICATION

This is to certify that this research project titled Evaluation Of the safety assessment of lysine producing Lactic Acid Bacteria and yeast isolated from *Ogi* a fermented sorghum gruel was carried out by Anyaokei Naomi Ifeoma (19/5771) under the supervision in the department of biological sciences and biotechnology college of pure and applied sciences in Caleb University, Imota, Ikorodu, Lagos state.

…… ……

DR. (MRS.) O. A. FOLORUNSO DATE

PROJECT SUPERVISOR

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DR. (MRS.) C. C. EZEANYA-BAKPA DATE

HEAD OF DEPARTMENT

…….. ……..……

EXTERNAL EXAMINER DATE

DEDICATION

I dedicate this project to the Almighty God for His grace in my life and for making this a success, and also to my irreplaceable mum and grandparents Miss Jibunoh and Mr. and Mrs. Jibunoh for their ceaseless care and support throughout this journey.

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ABSTRACT

Ogi is a traditional Nigerian food product derived from cereals like maize, sorghum, and millet. One of the major sources of bacterial contamination of *Ogi* production is bad water coupled with unhygienic conditions of processing and handling associated with indigenous processes. Lysine is one of the essential amino acids required for human and animal growth. Microbial food safety is a public health concern and fresh *Ogi* is known to house an array of Lactic acid bacteria such as *Lactobacillus plantarum* which are fermenters. This study was carried out to evaluate or determine the safety assessment and how poor production of *Ogi* is detrimental to human health. Since the relevance of these fermenting organisms during fermentation is indispensable, it is paramount that the safety assessment to the use of these organisms as starter cultures in the production of ogi and other fermented foods is evaluated. Morphological tests (cellular and colony morphology), physiological tests (growth at different pH and temperatures), and biochemical tests (catalase, motility, citrate) were carried out to further distinguish and confirm the Lactic Acid Bacteria (LAB) and yeast organisms that were suspected. For the purpose of my study, evaluation of safety assessment was carried out this includes; gelatinase production, DNase production and hemolysis production. Six LAB and yeast were isolated from traditionally made *Ogi* a fermented sorghum gruel. They isolates were identified and characterized based on some morphological, physiological, and biochemical tests. Some of the species includes; *Lactobacillus bulgaricus, Lactobacillus casei, Saccharomyces cerevisae, Candida famata* etc. In conclusion, the safety assessment of lysine producing LAB and yeast isolated from *Ogi,* a fermented sorghum gruel was evaluated in this study. Thus, safety assessment of the lysine producing LAB and yeast isolated from *Ogi* will help in optimizing the application and the performances of the microorganisms for better food safety and related products.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Ogi is a traditional Nigerian food product derived from cereals like maize, sorghum, and millet. However, *Ogi* from maize is more popular compared with other cereals. Maize (*Zea mays*) is one of the most important cereals in the world (Izah *et al.,* 2016). A large portion of it, carbohydrates, are stored as starch (Okeke *et al.,* 2018). In most African countries, some maize products are fermented foods that constitute many infants and adults (Okeke *et al*., 2015; 2018). It is produced generally by soaking maize grains in warm water for 2-3 days followed by wet milling and sieving through a screen mesh. The chemical composition of *Ogi* blends showed that moisture content ranged from 7.45%-10.15%, ash between 0.22-0, and fat content ranged from 2%-5.9%. Protein content ranged from 5.73%-7.43% while carbohydrate ranged from 77.00%-81.46% (Omole *et al.,* 2017). It has also been shown that *Ogi* liquor has both anti-bacterial (Bolaji *et al.,* 2017) and antifungal properties (Jude-Ojei *et al.,* 2017).

Lysine is one of the essential amino acids required for human and animal growth. Lysine and other amino acids are commonly produced by fermentation using strains of heterotrophic bacteria, such as *Escherichia coli* and *Corynebacterium glutamicum* (Lim *et al*., 2019), and synthesized from yeast such as *Saccharomyces cerevisiae* (Özogul and Hamed, 2018). The human body cannot synthesize lysine. It is essential in humans and must be obtained from the diet. In organisms that synthesize lysine, it has two main biosynthetic pathways, the diaminopimelate, and α-aminoadipate pathways, which employ distinct enzymes and substrates and are found in diverse organisms (Omole *et al.,* 2017). Lysine catabolism occurs through one of several pathways, the most common of which is the Saccharopine pathway (Pena *et al*., 2017; Li *et al.,* 2019).

The microorganisms responsible for the fermentation of maize for *Ogi* production play an important role in aroma, microbial stability, and flavor. Previous studies have concentrated mainly on the role of the lactic acid bacteria and their use as starter cultures in *Ogi* production. Yeasts have been reported to be involved in several different types of indigenous fermented foods and beverages and an overview of their possible functions in African indigenous fermented foods and beverages has been reported (Ayivi *et al.,* 2020). Important enzymatic qualities with nutritional effects had been reported in many lactic acid bacteria and yeasts in indigenous fermentation. These enzymes allow the breakdown of many complex substances such as starch, oligosaccharides, protein, and phytic acid complexes thus increasing the quantities and qualities of easily digestible nutrients in foods. Important enzymatic qualities with nutritional effects had been reported in many lactic acid bacteria and yeasts in indigenous fermentation. These enzymes allow the breakdown of many complex substances such as starch, oligosaccharides, protein, and phytic acid complexes thus increasing the quantities and qualities of easily digestible nutrients in foods. This feature is of major benefit to the consumers of *Ogi,* the majority of who are infants, children, and convalescent adults with weak digestive systems (Liu *et al.,* 2014; Azam *et al.,* 2017).

Lactic acid bacteria mainly *Lactobacillus plantarum* and *Lactobacillus fermentum,* and the yeast *Saccharomyces cerevisiae* and *Pichia kudriavzevii* are the predominant microbial species in the fermentation of cereals for the production of *Ogi* (Tope,2014; Adebolu *et al.,*2018). The majority of the microbes in fermented steeped maize for *Ogi* production have been variously reported to participate in the fermentation of some other local food. These microorganisms are present in the environment and can inoculate suitable substrates at will (Tope, 2014; Adebolu *et al.,* 2018).

1.2 Statement of Problem

The goal of this study is to determine the safety of lysine producing LAB and yeast isolated from *Ogi.* Despite the precarious health of some Ogi consumers and the widespread use of lysine in dietary supplements and foods, little is known about the role of the microorganisms in the production of lysine as well as their potential to produce harmful metabolites. Effective parameters for monitoring lysine producing lactic acid bacteria and yeast are also required to determine when a fermentation process should be terminated to avoid the growth of any harmful metabolites.

1.3 Aim and Objectives

Aim of Study

To evaluate the safety assessment of lysine-producing LAB and yeast isolated from *Ogi*.

Objectives

i. To isolate and characterize the lysine-producing lactic acid bacteria and yeast isolated from *Ogi*.

ii. To screen for lysine-producing LAB and yeast from *Ogi*.

iii. To evaluate the production of hemolysis, gelatinase, and DNase in lysine-producing lactic acid bacteria and yeast isolated from *Ogi*.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Ogi*

Ogi is a staple cereal fermentation product found predominantly in Southern Nigeria and is usually the first native food given to babies at weaning. It is produced generally by soaking maize grains in warm water for 2-3 days followed by wet milling and sieving through a screen mesh (Chilaka *et al*., 2016). Olaniran and Abiose (2018) reported the use of *Ogi* as a weaning food in western Nigeria to supplement breastfeeding between the ages of 3-6 months. However; this may be inadequate to meet the nutritional demands of growing infants. It has also been shown that *Ogi* liquor has both antibacterial (Adebolu *et al*., 2007) and antifungal properties (Adebayo and Aderiye, 2010). *Ogi* is usually prepared from fermented maize, sorghum, or millet in West Africa (Afolabi *et al.*, 2018). It is a popular breakfast cereal and infant weaning food in Nigeria (Omole *et al.*, 2017; Afolabi *et al*., 2018). It can be diluted into solids content of 8 to 10% and boiled into a pap, or cooked and turned into a stiff gel called *agidi* or *eko* before consumption (Bolaji *et al*., 2019). This same product is often eaten along with meat, stew, vegetable soup, steamed bean cake (*moin-moin*), or fried bean cake (*akara*) (Bolaji *et al*., 2019). The economic strength of the consumers does influence the choice of supplements (Ademola *et al.*, 2021). It has been established that substantial nutrient losses occur during the various stages of production of *Ogi*. These losses have been evaluated and reported by several workers (Awoyale *et al*., 2016; Ojo and Enujiugha, 2016; Folakemi *et al*., 2019).

2.2 Characterization and Production of *Ogi*

Several cereals can be employed for the production of *Ogi* either independently or in combination. Maize, sorghum, and millet are the major cereals used as substrates for the production of *Ogi* (Chilaka *et al.*,

2016). Millet seeds are rich in phytochemicals and phytic acid which is believed to lower cholesterol, phytate on the other hand is linked to cancer reduction (Achi and Ukwuru, 2015).

The traditional technique of *Ogi* fermentation has been extensively studied and involves spontaneous fermentation of the grains by soaking in water for about 48 hours at 28 ± 2°C and milling into a smooth paste. The slurry obtained is then sieved using a muslin cloth to remove the bran, germ, and hull which is high in protein. The filtrate is allowed to undergo secondary fermentation for about 24–72 h to develop its characteristic sour taste. The length of the secondary fermentation depends on the extent to which sourness is desired. In another context, the grains are soaked in hot/warm water for about 12-24 h before fermentation to facilitate softening of the cotyledons (Afolayan *et al.*, 2017). This method is usually practiced by the traditional unskilled producers who lack adherence to good manufacturing practices and sanitation, especially in rural areas where potable water supply is a major concern. These unsanitary practices expose the product to not only contamination from handling and processing, but water-borne pathogens. The traditional method of *Ogi* fermentation is labor-intensive and time-consuming (Adisa *et al*., 2019).

2.3 Chemical Properties of *Ogi*

2.3.1 Physicochemical Properties

Studies on pH and titratable acidity (TTA) of the fermented slurry from different grains revealed that as the TTA increases, the pH value decreases (Bolaji *et al*., 2014). This trend was also observable during starter culture fermentation of *Ogi* production (Farinde *et al*., 2014). The reason for this decrease in pH is due to the presence and activities of lactic acid bacteria (LAB) which resulted in the production of lactic acid during *Ogi* fermentation (Ojo and Enujiugha, 2016). Antimicrobial and bacteriocins which are of great value in bio-preservation and improved product shelf life by eliminating spoilage and pathogenic organisms have also been linked to the low pH of fermented foods (Bolaji *et al*., 2015). During

fermentation of *Ogi*, some of the fermenting organisms produce amylolytic enzymes which are responsible for the disintegration of the starch substrate to reduce sugars, thereby resulting in the decrease in the total sugar content of the *Ogi.* In addition, lowering the pH is also affected by the ability of yeast and LAB present during fermentation to utilize the free sugars. Another point of note is the increase in bacteria counts which increases as steeping progress favoring the growth of lactic acid bacteria, thereby increasing the acidity of the steeped water and *Ogi* at the end of fermentation (Farinde, 2015).

2.3.2 Proximate Composition, Functional and Nutritional Contents of *Ogi*

Several studies have reported considerable variation in the proximate composition of maize, sorghum, and millet *Ogi* (Folakemi *et al*., 2019), with the agro-climatic condition under which these grains are cultivated showing a significant influence on the composition of the grains and the products obtained from them (Adisa *et al*., 2019). *Ogi* produced using sorghum had the highest protein content followed by maize *Ogi* which varied with the result of (Bolaji *et al*., 2015), while millet ogi had the highest protein content (7.92%) followed by sorghum *Ogi* (5.93%) (Afolayan *et al*., 2017).

The study conducted by (Bolaji *et al*., 2014) reported that after 72 h of fermentation, the proximate composition showed higher carbohydrate content (19.31%) than the unfermented cornflour (11.14%). However, the protein, fiber, fat, and ash content were observed to be higher in the unfermented cornflour (11.90%), (4.73%), (1.30%), (3.45%) respectively compared with the fermented corn slurry (3.33%), (0.15%), (0.13%) and (1.75%). The loss of protein in the fermented sample is to the findings of (Arifin *et al*., 2014) which showed that the stages involve in the production process of grains to ogi resulted in protein loss of about 40% however, it increased the digestibility by about 20%.

Functional properties of *Ogi* samples from different varieties of grain have been reported to show variation, this trend was observed for water absorption capacity, swelling power, solubility, bulk density, and pasting characteristics (Bolaji *et al.,* 2014). This variation has been linked with the ratio of the

amylose to amylopectin components of grain, and the characteristics of each fraction in terms of molecular weight, distribution, length of branching, and conformation. High bulk density is desirable, to reduce the paste thickness which is an important factor in convalescent and child feeding (Laduni *et al*., 2015).

2.4 Microbiological Properties of *Ogi*

The traditional fermentation is initiated as a result of chanced inoculation by uncontrolled microorganisms from the environment involving a build-up of bacteria and yeasts. Some of these microorganisms may participate in parallel, while others act in succession with a changing dominant biota during fermentation. studies have been able to isolate and enumerate possible microorganisms associated with the fermentation of *Ogi* (Gadaga *et al*., 2001; Jespersen, 2003). The following genera predominate the bacterial fermentation, *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Micrococcus,* and *Bacillus* The yeasts genera are included representatives of *Saccharomyces*, *Candida*, *Aspergillus*, *Fusarium*, *Cladosporium* and *Penicillium* amongst others Lactic acid bacteria (LAB) are one the most common microorganisms responsible for cereal fermentations, they are notable for the beneficial role of preservation, enhanced nutritional value, detoxification, lactic acid, flavor and aroma production with *Lactobacillus plantarum* reported as the most dominant specie (Bolaji *et al*., 2015). These organisms have been studied to competitively eliminate other organisms, especially pathogens from the fermentation process. The synergy between LAB and yeast is common in food and beverage fermentations with LAB creating the acidic environment for yeast growth and yeast providing the vitamins and other growth factors necessary for the survival of Lab (Gadaga *et al*., 2001; Jespersen, 2003). Lactic acid bacteria (*Lactobacillus plantarum* and *Streptococcus lactic*) and yeasts (*Saccharomyces cerevisiae, Rhodotorula* spp.*„ Candida mycoderma,* and *Debaromyces hansenii*) are predominantly involved in the fermentation of *Ogi*, playing important roles as aroma development,

microbial stability, and flavor enhancement (Aworh, 2008; Omemu *et al*., 2011). Lactic acid fermentation also plays important role in reducing antinutritional factors, increasing nutrient density, and antimicrobial activities in the fermented product (Oyarekua, 2013)**.**

2.5 Safety Assessment of Lysine-Producing Lab And Yeast In *Ogi* Production

Microbial food safety is a public health concern and fresh *Ogi* is known to house an array of lactic acid bacteria such as *Lactobacillus plantarum, Lactobacillus fermentum,* and *Streptococcus lactis* which are health-promoting food-grade organisms usually common with fermented foods (Sanlier, 2009). One of the major sources of bacterial contamination of *Ogi* is water coupled with unhygienic conditions of processing and handling associated with the indigenous processes. This is particular to communities where potable water is not available leading to frequent heavy contamination of the product with pathogens and may be a major factor in causing food-borne illnesses such as diarrhea and associated malnutrition in children (Karabudak *et al*., 2008). For day-to-day living, individuals ingest a substantial portion of yeasts without antagonistic effect on their wellbeing. Yeasts are hardly related to occurrences of foodborne diseases, food poisoning, or infections, unlike bacteria and viruses. Nonetheless, care is necessary, as well as more investigation regarding the use and application of yeast (Fleet and Balia, 2006).

Yeasts exist as non-antagonistic, infective organisms, but a few species live as opportunistic disease-causing agents responsible for a variety of infections (Hazen and Howell, 2003). Persons with deteriorated well-being are vulnerable to these infections. Increasing news report about yeast infections is a result of the increasing proportion of such persons in the community. Moreover, the figure of yeast species found present in food materials is on the increase (for example, *Candida krusei, Saccharomyces cerevisiae, Pichia anomola, Candida format,* and species of *Rhodotorula*) (Hobson, 2003; Fleet and Balia, 2006). There have been notable infections caused by *S. cerevisiae* in immune-compromised persons (de Llanos *et al.,* 2006). Hospitalized patients are believed to become imperiled to elevated concentration of

yeasts via the biofilms created on the waste pipe and other invasive machines, contact with yeasts may probably come from the body of workers in the hospital as well as the meals are taken into the environment around the hospital (Fleet and Balia, 2006).

Therefore, there is a need to build more effective relationships amid the functionality of fares in supporting yeast-related disease through more investigations. Also, detailed information is necessary to know the existence and development of yeasts in the GIT into the circulatory system, and of course, the common existence of yeasts in environs of medical centers. Furthermore, it is important to investigate the cases in which a non-causing disease agent, for instance, *Saccharomyces cerevisiae* turns out to be disease causative (Fleet, 2007).

2.6 Probiotic Attributes of *Ogi*

Probiotics have been linked to fermentation as beneficial living microorganisms that, when consumed in sufficient quantities (106 cfu/ml), confer health benefits on the host (Olukoya *et al.,* 1994). Probiotic bacteria have properties such as acid and bile resistance, the ability to produce antimicrobial substances, adherence to epithelial tissue, colonization of the gastrointestinal tracts, stimulation of the host immune response, cholesterol, and lactose activity. *Lactobacillus* species have been employed as probiotics. For example, *Lactobacillus acidophilus* has been exploited as an important probiotic since it is known to be the dominant LAB. However, a wide variety of lactobacillus has been utilized in preparing probiotics consisting of diverse species of *Lactobacillus* (Steintrause, 1995; Vinderola *et al.,* 2002). Therefore, the use of LAB as probiotics necessitates a good and safe evaluation. Each strain's useful and effective characteristics have been reviewed and recorded thoroughly (Holzapfel *et al.,* 2001). The potential of probiotics to remain in *Ogi* and modification of immune responses are largely accepted as health-stimulating and non-pathogenic properties (Reid *et al.,* 2003). The significance of probiotic consumption, for example, bulging and obstructing the gastrointestinal tract has been investigated (Gibson and Fuller,

2000). Bacteria could alter the constituent of food or organic secretions transforming these materials into other secondary constituents which may be harmful to the host (Ishibashi and Yamazaki, 2001).

LAB is considered majorly a class of healthy microbes (Collins *et al*., 1998). Marteau *et al.* (1995) described probiotics to mean 'for life' as microorganisms have been confirmed to offer medical benefits in humans and animals. Steinkrause (1995) viewed probiotics as active microorganisms, beneficial to the body of the host by enhancing the stability of microorganisms in *Ogi*. Similarly, Agerholm-Larsen *et al.* (2000) proposed that probiotics are living microbes ingested to provide medical benefits, furthermore offering qualitative nutritional balanced diet, they communally conserve intricate stability between the gastrointestinal area and defense systems whereas prebiotics is termed as food constituents which foster increased development as well as activities of important abdominal bacteria, hence obtaining awareness and significance as useful foods quickly.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Red Sorghum grains were purchased at Lawanson market in Surulere Lagos state and were transported in clean nylon to the laboratory for the preparation of *Ogi.*

3.2 Laboratory production of *Ogi*

The preparation of sorghum *Ogi* was done according to the method described by Adisa *et al.,* (2019) where by red sorghum grains were soaked in water for 72 hours. After 72 hours, the sorghum grains were ground into a smooth paste. The resulting slurry was sieved using a muslin cloth to remove the chaff. The mixture of water and *Ogi* was set aside to settle for at least three hours. After about three hours, clear water was seen, the clear water was decanted and the rest of the mixture was poured into muslin cloth. The muslin cloth was tied to allow water drain from the *Ogi* for 24 hours. After draining the water, the *Ogi* was brought out of the bag ready for use.

3.3 Culture Media Preparation

The media used was MRS (De Mann Ragosa Sharpe) Agar and MEA (Malt Extract Agar), the agar was measured and mixed in distilled water following the manufacturer's instructions. After preparing the agar, it was autoclaved at 121℃ for 15 minutes, and allowed to cool to 45℃ before use. After cooling the agar, it was placed into different marked Petri dishes and left to solidify.

3.4 Isolation of LAB and Yeast from Laboratory-Prepared *Ogi*

The isolation of yeast from sorghum *Ogi* was done according to the method described by Harrigan and McCane *(*1976) whereby the pour plate technique was used, serial dilution was made from laboratory-prepared *Ogi* using a sterile pipette. This was done by mixing 1ml of the prepared sample thoroughly

with 9ml of sterile distilled water to give e 1:10 dilution. The dilution was made up to 10^{-6}. Using a sterile pipette of 1ml, appropriate dilution was plated out using different culture media. The plates were inoculated in duplicates and allowed to set. After solidifying the plates were inverted. At the end of incubation, representative colonies with the obvious morphological difference such as color, size, and shape were randomly picked from MRS agar plates and malt extract agar plates as LAB and yeast isolates respectively.

3.5 Identification and Characterization of LAB and Yeast from Laboratory Prepared *Ogi*

All isolates were sub-cultured to obtain a pure culture. Bacteria and yeasts were tentatively identified based on physiological and biochemical features.

3.6 Morphological Characterization of LAB and Yeast Isolates

3.6.1 Colonial Morphology

The colony morphology (color, size, form, surface, and opacity) of each isolated LAB and Yeasts was observed and recorded.

3.6.2 Cellular Morphology

- **Gram Staining**

Gram staining was carried out to determine the cellular characteristics of isolates. A loop full of freshly prepared LAB isolate was taken using a sterile wire loop and placed on a clean slide. This is followed by the addition of crystal violet for 1min and rinsed. This is followed by the addition of grams iodine for 1 min and then add alcohol for 30 seconds and then rinsed with water. The addition of safranin was added for 1 min and rinsed with water. The slide was left to dry. Microscopic examination of the slides was done with a microscope using the oil immersion (×100) lens.

3.7 Physiological Characterization of LAB and Yeast Isolates

3.7.1 Growth at different pH

A total of 500mL of MRS broth was divided into two beakers and the pH of the media was adjusted to 3.9 and 9.6 using diluted HCL. Freshly prepared LAB isolates were inoculated into the pH-adjusted MRS broth and incubated anaerobically for 3-5 days. Turbid tubes showed a positive result while unturbid tubes showed negative results and the control remained unturbid.

3.7.2 Growth at Temperatures of 15°C and 45°C

MRS broth plates were prepared and isolates were inoculated and incubated for five days at temperatures of 15℃ and 45 ℃ respectively. The isolates were incubated anaerobically at a temperature of 15℃ were kept in the refrigerator while isolates of 45℃ were incubated in the oven.

3.7.3 Growth at 4% NaCl

About 100ml of MRS broth prepared with 4% NaCl was added to 500ml of agar and sterilized at 121℃ for 15 minutes and then inoculated with fresh cultures of LAB and yeast isolated and incubated for 2-3 days. After cooling the agar containing 4% NaCl was poured into a sterile petri dish and the isolates were inoculated with a sterile inoculating loop on the agar plate to determine growth and waswerecubated for 48 hours (Meconi *et al.,* 2014).

3.8 Biochemical Characterization of LAB and Yeast Isolates

3.8.1 Catalase Test

The catalase test was carried out by transferring a small amount from freshly prepared LAB isolates directly to a slide using an inoculating loop. Then one drop of hydrogen peroxide was dropped on the small amount of the organism that was transferred to the slide. A bubble formation indicated a positive result while no indication of bubbles showed a negative result.

3.8.2 Potassium Hydroxide (KOH_3)

About 0.3g of KOH was weighed and dissolved in 10ml of water. A drop of the KOH solution was then placed on a slide, with a flamed inoculating loop, and freshly prepared LAB isolates were then mixed with the solution. Mucoid formation showed negative results while non-mucoid formation should have positive results.

3.8.3 Motility Test

According to Onyeze *et al.* (2013), this test is carried out to determine the presence or absence of flagella as a motility organelle in the LAB isolates. Freshly prepared LAB (18-24 hours) was placed on a microscopic slide containing a drop of peptone water and covered with a coverslip after a minute. Then it was examined under a microscope with high-power objectives.

3.8.4 Indole Test

According to Onyeze *et al.* (2013), freshly prepared LAB isolates were inoculated using a sterile wire loop into a test tube containing 5ml of peptone water (medium) and incubated for 48hours at 37℃. After incubation, the tube was filled with 0.5ml of Kovac's reagent and let to stand for 15 minutes.

3.8.5 Methyl Red Test

According to Onyeze *et al.* (2013), freshly prepared LAB isolates were inoculated in a test tube containing 5ml peptone water and were incubated for 48 hours at 37℃. After incubation, 0.5ml of methyl red was added to the test tubes and allowed to stand for 15 minutes.

3.8.6 Voges Proskauer Test

According to Tankeshwar *et al.* (2013), freshly prepared LAB isolates were inoculated in a test tube containing glucose-phosphate broth and incubated at 35℃ for 24 hours. After incubation 1ml of the glucose-phosphate broth was suspended in a clean test tube, followed by the addition of 0.6ml of 5%

naphthol and 0.2ml of 40%. The tube was left to cool for about 10 to 15 minutes after shaking it gently to expose the medium to atmospheric oxygen.

3.8.7 Sugar Fermentation Test

According to Akin-Osanaiye and Kamalu (2019), In each sterile test tube, exactly 10ml of peptone water was added. Each of the test tubes containing peptone water had one gram of the corresponding carbohydrates, such as D-glucose, sucrose, mannitol, fructose, galactose, lactose, xylose, and maltose, added and labeled accordingly. Warming them over a Bunsen burner allowed them to disintegrate. The basal medium was Bromocresol purple broth foundation. A 1% filter-sterilized sugar solution was added aseptically to sterilized bromocresol purple broth base using a 0.2m Millipore filter (corning). The tubes were sterilized in an autoclave at 115°C for 15 minutes after being plugged with cotton wool and sealed with foil. The cultural organisms were introduced into each of the tubes after the medium had been sterilized. After 5 days of anaerobic incubation at 30°C, the findings were compared to an uncoated control. The use of sugar or acid production was indicated in tubes where the bromocresol purple color turned to yellow. The appearance of bubbles on the medium's surface and the upward movement of the inverted Durham tubes indicated gas production.

3.8.8 Citrate Test

The Citrate test was done to determine the ability of the isolates to utilize citrate as their sole source of carbon and ammonia as their sole source of nitrogen. Simmons citrate was prepared and homogenized in a water bath. It was then placed in cryovial tubes and autoclaved. After sterilization, it was slanted and allowed to solidify. It was then inoculated with 18-24 hours LAB isolate and incubated at 37^0C for 48 hours. Color change from green to deep blue indicated positive results while no color change indicates a negative result control tubes did not show any color change.

3.8.9 Bile Salt Assay

The assay medium used contained MRS agar supplemented with 0.5% of bile salt. For 72 hours, the plates were incubated anaerobically at 37℃. The presence of a clear zone around the colonies showed a positive reaction.

3.8.10 Arginine Analysis

According to Sirisha *et al.,* 2021, freshly prepared LAB isolates were inoculated in arginine broth to test for the production of ammonia from arginine. 100µl of the sample was spotted on a white tile after 24 hours of incubation and an equivalent volume of Nessler's reagent was added. The presence of ammonia as a result of arginine hydrolysis is indicated by the immediate emergence of the dark orange color.

3.8.11 CO_2 Production from Glucose

According to Akweya *et al*. (2022), this test is used to determine the ability of the LAB isolates to produce gasses and acid. In test tubes containing inverted Durham tubes, overnight sample cultures were inoculated into 8ml of MRS broth. The tubes were then anaerobically incubated for 48 hours at 37℃ in an anaerobic jar. After incubation, gas evolution in Durham tubes was shown by the space produced in the tube showing the production of co_2 from glucose which shows they are hetero-fermenters and no gas production indicated the absence of air bubbles signifies that they are homo-fermenters.

3.9 Screening for Lysine-Producing LAB and Yeast Isolated from *Ogi*

According to Okpala *et al.* (2019) and Chike-Mozie *et al.* (2015), the isolates were tested for lysine synthesis using a minimal medium containing glucose, 4.0g; $(NH_4)\ 2SO_4$, 2.0; K_2HPO_4, 0.5g; $MgSO_4.7H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $MnSO_4.4H_2O$, 0.001g; $CaCO_3$, 2g; Agar, 15.0g; and water, 1 liter, with PH adjusted in a 250 ml Erlenmeyer flask, was sterilized at 115℃ for 15mins, allowed to cool to 40℃. The molten agar medium was placed into Petri plates that had been seeded with a 24-hour

broth culture of the lysine auxotroph, *Escherichia coli* solidified and then inoculated with the isolates. As a control, an uninoculated agar plate was used. (Okpalla *et al.,* 2019). After 96 hours of incubation at 30℃, the plates were examined for halo growth of the *Escherichia coli* auxotroph, which indicates that the isolates are producing lysine organisms.

3.10 Evaluation of the Safety Assessment of LAB and Yeast from Laboratory Prepared *Ogi*

3.10.1 Gelatinase Production

According to Afolake *et al.* (2019), Gelatinase production was assessed using Luria-Bertani (LB) agar containing gelatin (30g/l). The LAB and yeast isolates were grown overnight on Brain-Heart infusion agar plates at 37℃. One drop each of the colonies was inoculated onto the (LB) agar containing gelatin. After inoculation, the plates were incubated overnight at 37℃. A positive result shows a turbid appearance around the inoculated site, a negative result shows no turbid appearance around the inoculated site.

3.10.2 Dnase Production

According to Afolake *et al.* (2019), the LAB and yeast isolates were inoculated on the DNase agar which contains a pancreatic digest of casein (10g), yeast extract (10g), DNA (2g), sodium chloride (5g), agar (15g), methyl green (0.5g), water (200L). After inoculation, the plates are incubated at 35-37℃ for 24 hours. A positive result indicates a colorless medium around the inoculum while a negative result if no degradation of DNA occurs, the medium remains green.

3.10.3 Haemolysis Production

According to Afolake *et al.,* 2019, freshly prepared LAB and yeast isolates were inoculated onto a sterile blood agar plate at 30℃ for 24 hours. After incubation overnight, the medium is inspected for signs of alpha, beta or gamma hemolysis. An alpha hemolysis is indicated by a discolored medium, beta

hemolysis is indicated by a cleared undergrowth medium and gamma hemolysis is indicated by no desired color change of the medium.

CHAPTER FOUR

RESULTS

In this study, six species of LAB were isolated from traditionally made *Ogi* a fermented sorghum gruel. The LAB isolates were identified and characterized based on morphological tests, physiological tests, and biochemical tests and identified according to Bergey's manual.

The LAB species were identified as *Lactobacillus acidophillus, L. bulgaricus L. casei, L. plantarum, L. fermentum, L.brevis*. Morphological, physiological and biochemical characteristics of the LAB isolates. The cell and colony morphology carried out showed that all LAB isolates from fermented *Ogi* were cream colored, Gram positive, non-motile, non-spore forming rods. Most of the isolates were short rods while *L. bulgaricus* was the only elongated rod. The physiological characteristics of the LAB isolates revealed that all the LAB isolates fermented glucose and all the isolates except from *L. brevis* fermented fructose, most LAB isolates grew at 15℃, most LAB isolates grew at 45℃ with *L. fermentum* showing no growth at 45℃. Most isolates were able to grow at 4% Nacl, all the LAB isolates grew at PH 3.9 and 9.6 respectively. The biochemical characteristics of the LAB isolates revealed that all the LAB isolates were negative to catalase, oxidase, motility and indole test. All the LAB isolates tested positive to glucose test, most of the isolates were positive to maltose with *L. fermentum* being negative, most of the isolates were positive to mannitol, sucrose and fructose fermentation test with *L. bulgaricus and L. brevis* being negative (Table 4.1).

In this study, six species of yeast were isolated from *Ogi* a fermented sorghum gruel. The isolated *Candida* organism was identified and characterized based on their morphological tests, physiological tests, biochemical tests, and sugar fermentation tests according to mycology manual. The yeast species are identified as; *Saccharomyces cerevisiae, Candida parapsilosis, tropicalis, Candida famata, Candida utilis, Hansenula anomala.*

Morphological characteristics of yeast isolated from *Ogi* a fermented sorghum gruel the colony morphology revealed the isolates were creamy, small-sized and smooth, and the cell morphology showed that the isolates were mostly small, oval shaped cells (Table 4.2).

Physiological characteristics of yeast isolated from *Ogi* a fermented sorghum gruel revealed that a variety of the yeast isolates grew at temperatures of 15℃ and 45℃ respectively, the isolates grew at PH concentration of 2.0 and 3.0 (Table 4.3).

Biochemical characteristics of yeast isolated from *Ogi* a fermented sorghum gruel and it reveals that the yeast isolates had variation in the fermentation of glucose, maltose, fructose, mannitol, sucrose, galactose, lactose and xylose. All the yeast isolates showed negative to motility test and all the isolates showed positive reaction to catalase test. All the isolates revealed a negative reaction to oxidase and indole test with an exception of *Candida tropicalis* which was positive to oxidase test (Table 4.4).

The screening of lysine producing LAB was isolated from *Ogi* using a minimum medium containing glucose, potassium hydrogen phosphate, diazanum sulfate. Six isolates were screened for lysine and the result revealed that most of the isolates were positive to lysine with *L.homonis* being the only negative lysine producer (Table 4.5).

The screening of lysine producing yeast was isolated from *Ogi* which was carried out on a solid agar medium seeded with lysine auxotroph E. *coli.* six yeast isolates were screened for lysine and all the isolates were positive to the screening (Table 4.6).

The evaluation of DNase hydrolysis, gelatinase hydrolysis and hemolysis of lysine producing LAB isolated from *Ogi* a fermented sorghum gruel. For DNase hydrolysis, *Lactobacillus canosus, Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus curvatus, Lactobacillus brevis,* and *Lactobacillus casei* were recorded as positive. *Lactobacillus delbrueckii, Lactobacillus bulgaricus,*

Lactobacillus curvatus, Lactobacillus buchneri, and *Lactobacillus plantarum* was recorded as negative. For Gelatinase hydrolysis test, *Lactobacillus fermentum, Lactobacillus curvatus Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus bulgaricus,* and *Lactobacillus planetarium* were positive. *Lactobacillus canosus, Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus bruchneri,* and *Lactobacillus casei* were recorded as negative. During hemolysis, Lactobacillus *brevis* was the only positive organism The other species were recorded as negative (Table 4.7).

The evaluation of DNase hydrolysis, gelatinase hydrolysis and hemolysis of lysine producing yeast isolated from *Ogi* a fermented sorghum gruel. For DNase hydrolysis *Candida tropicalis,* and *Saccharomyces baili* were both positive while *Geotrichum capitatum, Candida sphaerica, Hansenula anomala, Geotrichum candidium, Candida krusei Saccharomyces cerevisae, Candida valida, Candida albican, Candida parapslosis, Candida famata, and Candida utilis* were negative. For gelatinase hydrolysis, *Geotrichum candidium, Candida krusei, Saccharomyces cerevisiae, Candida valida, Hamsenula anomala, Saccharomyces baili,* and *Candida parapslosis,* were positive. While the other species of yeast were negative. For hemolysis, only *Candida albicans* was positive. The other organisms were negative for hemolysis and were recorded as gamma hemolysis (Table 4.8).

Table 4.1: Morphological, Physiological, and Biochemical Characteristics of LAB Isolated from *Ogi,* a fermented sorghum gruel

Isolate Code	Nh_3 From Arginine	Catalase	Oxidase	Motility	Indole	MR	VP	Casein	Starch	Cellular Morphology	Colony Morphology	Gram Reaction	Growth At 15°c	Growth At 45°c	Growth At 3.9 Ph	Growth At 9.6 Ph	Growth At 4% Nacl	Growth In Bile only	Glucose	Maltose	Mannitol	Sucrose	Fructose	Probable organism
LB05	-	-	-	-	-	+	-	+	+	Short rods	C	+	-	+	+	+	+	+	+	+	-	+	+	*Lactobacillus acidophillus*
LB06	+	-	-	-	-	+	-	+	+	Short rods	C	+	+	+	+	+	-	+	+	-	-	+	+	*Lactobacillus fermentum*
LB17	+	-	-	-	-	+	-	+	+	Short rods	C	+	+	-	+	+	-	-	+	+	+	+	-	*Lactobacillus brevis*
LB21	-	-	-	-	-	+	-	+	+	Elongated rods	C	+	-	+	+	+	-	-	+	-	-	-	-	*Lactobacillus bulgaricus*
LB26	-	-	-	-	-	+	-	+	+	Short rods	C	+	+	-	+	+	+	+	+	+	+	+	+	*Lactobacillus casei*
LB31	-	-	-	-	-	+	-	+	+	Short rods	C	+	+	-	+	+	+	-	+	+	+	+	+	*Lactobacillus plantarum*

Keys: MR: methyl red, VP: Voges Proskauer test, +: Positive, -: Negative, C: cream

Table 4.2: Morphological Characteristics of Yeast Isolated from *Ogi,* a fermented sorghum gruel

Isolate code	Colony morphology	Cell morphology	Probable organism
Y07	Creamy, small-sized, round, flat, smooth, opaque colony	Oval-shaped, small clustered	*Hansenula anomala*
Y26	Creamy, flat, round, smooth, raised, glossy, entire, opaque colony	Small clusters, oval-shaped	*Candida parapsilosis*
Y30	Creamy, medium-sized, round, flat, smooth, raised, undulating colony	Small clusters, oval-shaped	*Saccharomyces cerevisiae*
Y33	Creamy, round, small-sized, smooth colony	Small, oval-shaped	*Candida tropicalis*
Y34	Creamy, round, flat, smooth, entire, opaque colony	Small clusters, oval-shaped, elongated	*Candida famata*
Y42	Creamy, small- sized, round, flat, smooth colony	Small clusters, oval-shaped	*Candida utilis*

Key: Y: Yeast

Table 4.3: Physiological Characteristics of Yeast Isolated from *Ogi,* a fermented sorghum gruel

Isolate code	15°C	45°C	pH 2.0	pH 3.0	Starch hydrolysis	KNO_3	Probable organism
Y07	+	+	+	+	+	-	*Hansenula anomala*
Y26	+	+	+	+	+	-	*Candida parapsilosis*
Y30	+	+	+	+	+	+	*Saccharomyces cerevisiae*
Y33	+	-	+	+	-	+	*Candida tropicalis*
Y34	+	-	+	+	+	+	*Candida famata*
Y42	+	+	+	+	+	-	*Candida utilis*

Key: Y: Yeast, , +: Positive, -: Negative

Table 4.4: Biochemical Characteristics of Yeasts Isolated from *Ogi*, a fermented sorghum gruel

Isolate code	Catalase	Oxidase	Motility	Indole	Glucose	Maltose	Fructose	Mannitol	Sucrose	Galactose	Lactose	Xylose	Probable organism
Y07	+	-	-	-	+	-	+	+	-	-	-	-	*Hansenula anomala*
Y26	+	+	-	-	+	+	+	+	+	+	-	+	*Candida parapsilosis*
Y30	+	-	-	-	+	+	+	-	+	+	-	-	*Saccharomyces cerevisiae*
Y33	+	+	-	-	+	+	+	+	-	+	-	+	*Candida tropicalis*
Y34	+	-	-	-	+	+	+	+	+	+	+	+	*Candida famata*
Y42	+	-	-	-	+	+	-	-	+	-	-	-	*Candida utilis*

Key: Y: Yeast, +: Positive, -: Negative

Table 4.5: Screening for lysine-producing LAB isolated from *Ogi,* a fermented sorghum gruel

Identified organism	Halo growth of *Escherichia coli*
Lactobacillus canosus	+
Lactobacillus homonis	-
Lactobacillus fermentum	+
Lactobacillus curvatus	+
Lactobacillus brevis	+
Lactobacillus bulgaricus	+

KEY; + Positive, -; Negative

Table 4.6: Screening for lysine-producing LAB isolated from *Ogi,* a fermented sorghum gruel

Identified organism	Screening results
Saccharomyces cerevisiae	+
Candida tropicalis	+
Hamsenula anomala	+
Candida famata	+
Candida parapsilosis	+
Candida utilis	+

KEY: +: Positive

Table 4.7: Evaluation of the DNase hydrolysis, Gelatinase hydrolysis, and hemolysis of lysine-producing LAB isolated from *Ogi,* a fermented sorghum gruel

Isolate code	DNase hydrolysis	Gelatinase hydrolysis	Hemolysis
LPL1	+	-	- γ
LPL2	+`	-	- γ
LPL3	+	-	- γ
LPL4	+	+	- γ
LPL5	+	-	- γ
LPL6	-	-	- γ
LPL7	+	+	- γ
LPL8	-	+	- γ
LPL9	-	-	- γ
LPL10	+	+	+ β
LPL11	+	+	+ β
LPL12	+	+	- γ
LPL13	-	+	- γ
LPL14	-	-	- γ
LPL15	-	-	- γ
LPL16	+	-	- γ
LPL17	-	-	- γ
LPL18	-	-	- γ
LPL19	-	+	- γ
LPL20	-	-	- γ

Key: LPL: lysine producing LAB, +; positive, -; negative, β; beta hemolysis, γ; gamma hemolysis

Table 4.8: Evaluation of the DNase hydrolysis, Gelatinase hydrolysis, and hemolysis of lysine-producing yeast isolated from *Ogi,* a fermented sorghum gruel in *Ogi*

Sample code	DNase hydrolysis	Gelatinase hydrolysis	Hemolysis
LPY1	-	-	- γ
LPY2	-	-	- γ
LPY3	-	-	- γ
LPY4	-	+	- γ
LPY5	-	+	- γ
LPY6	-	-	- γ
LPY7	-	+	- γ
LPY8	+	-	- γ
LPY9	-	+	- γ
LPY10	-	-	+ β
LPY11	-	+	- γ
LPY12	+	+	- γ
LPY13	-	-	- γ
LPY14	-	+	- γ
LPY15	-	+	- γ
LPY16	-	-	- γ
LPY17	-	-	- γ
LPY18	-	-	- γ
LPY19	-	+	- γ
LPY20	-	-	- γ
LPY21	-	-	- γ
LPY22	-	+	- γ
LPY23	-	-	- γ
LPY24	-	-	- γ

Key: LPY lysine producing yeast, + positive, - negative, β beta hemolysis, γ gamma hemolysis

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION

In this work, six lactic acid bacteria and six yeast species were isolated and characterized from traditionally fermented *Ogi* based on their morphological, physiological, and biochemical tests and was identified according to Bergey's manual and mycology's manual. Lactic acid bacteria and yeast can be found in a range of environments, including fermented foods. The LAB and yeast isolates were identified based on their Morphological, physiological, and biochemical test using Bergey's manual for LAB and mycology manual for yeast. The LAB were identified as *L. brevis, L. acidophillus, L. bulgaricus, L. fermentum, L. plantarum* and *L. casei.* The dominance of *Lactobacillus* fermentum in other Nigerian fermented foods was confirmed in the study of Sanni *et al*., (2002). This study's increased incidence of rod-shaped LAB supported a prior study (Adebayo-tayo and Onilude, 2008). (Sanni *et al*.,2002) also found that *L. plantarum* and *L. fermentum* strains were isolated at a high frequency of 24.6 and 26.3 percent, respectively, during *Ogi* fermentation. The yeast was identified as *Hansenula anomala, Candida parapsilosis, Saccharomyces cerevisae, Candida tropicalis, Candida famata amd Candida utilis* this agrees to the findings of Teniola *et al.,* (2001) who reported findings of similar yeast strains involved in the fermentation process of several foods especially *Ogi.*

A total 20 LAB isolates were screened for lysine and six isolates ranged from positive to negative, with *Lactobacillus canosus* being a lysine producer and *Lactobacillus homonis* being a non-lysine producer. Previous research has shown that *Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus curvatus,* and *Lactobacillus canosus* are highly adaptable species capable of colonizing a variety of habitats, including fermented grains, legumes, fruits, and meat (Banwo

et al., 2012; Adesulu-Dahunsi *et al*., 2020). Its presence in *Ogi* simply confirms its adaptability to a wide range of environments.

A total of 42 yeast isolated from sorghum *Ogi* were screened for lysine production on a solid agar medium seeded with lysine auxotroph, *Escherichia coli*. Six yeast isolates were observed to be active lysine producers, *Saccharomyces cerevisiae, Candida tropicalis, Hamsenula anomala, Candida parapsilosis, Candida utilis, Candida famata,* showed halo growth of the lysine auxotroph, *Escherichia coli* on the surface after incubation. *Escherichia coli* auxotroph being a mutant organism requires additional nutrient for its growth and ability to thrive.

The evaluation of the safety assessment of the lysine producing LAB and yeast using the parameters of DNase, gelatinase, and hemolysis were examined. *Lactobacillus canosus, Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus curvatus, Lactobacillus brevis,* and *Lactobacillus casei* were identified as positive result this may be attributed to the showed ability of the LAB to hydrolyse DNA, while *Lactobacillus delbrueckii, Lactobacillus bulgaricus, Lactobacillus curvatus, Lactobacillus buchneri,* and *Lactobacillus plantarum* showed as negative results because the organisms did not hydrolyse DNA, leaving no colorless zone around the medium and so keeping the medium's pale green color.

Geotrichum capitatum, Candida sphaerica, Hansenula anomala, Geotrichum candidium, Candida krusei Saccharomyces cerevisiae, Candida valida, Candida albican, Candida parapslosis, Candida famata, and *Candida utilis* were all positive because they could hydrolyze DNA, whereas *Geotrichum capitatum, Candida sphaerica, Hansenula anomala, Geotrichum candidium,* and *Candida krusei* were negative. After all, they could not hydrolyze DNA. *Lactobacillus fermentum, Lactobacillus curvatus, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus bulgaricus,* and *Lactobacillus planetarium* tested positive for the Gelatinase

hydrolysis test. This revealed the ability of the LAB to hydrolyse gelatin. Negative result to gelatin hydrolysis was seen in *Lactobacillus canosus, Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus bruchneri,* and *Lactobacillus casei* since the organisms are unable to hydrolyse gelatin. *Geotrichum candidium, Candida krusei, Saccharomyces cerevisiae, Candida valida, Hamsenula anomala, Saccharomyces baili,* and *Candida parapslosis* tested positive for gelatinase because the organisms were able to hydrolyze gelatin, which is a protein extract. While the other yeast species were negative due to their inability to hydrolyze gelatin. *Lactobacillus brevis* was the only positive bacteria that were able to lyse Red Blood Cells during the test of hemolysis hydrolysis. The results were also recorded as alpha hemolysis (partial hemolysis without transparency surrounding the colony), beta hemolysis (full hemolysis with transparency/clear zones around the colony), and gamma hemolysis (no hemolysis at all). Because they were unable to lyse the Red Blood Cells (erythrocytes), the other species were recorded as negative. Only *Candida albican* was able to lyse Red Blood Cells during the hemolysis examination, and it was reported as beta hemolysis because there was a distinct zone around the colony. The other organisms were not affected by hemolysis and were classified as gamma hemolysis.

CONCLUSION

Lysine-producing LAB and yeast were isolated from *Ogi* and the evaluation of the safety assessment was carried out. Some of the LAB isolates that tested positive for the safety evaluation includes; *Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus curvatus* while the yeast isolates *Candida albican, Geotrichum candidium, Candida krusei, Saccharomyces cerevisiae, Candida valida, Hamsenula anomala, Saccharomyces baili,* and *Candida parapslosis*. Thus, safety assessment of lysine producing lactic acid bacteria and yeast isolated from *Ogi* will help in optimizing the application and the performances of the microorganisms for improved food safety and related products. Controlled fermentation can be encouraged for upgrading the *Ogi* quality and consistency.

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