AMINO ACID PROFILE OF OGI PRODUCED USING LYSINE PRODUCING LACTIC ACID BACTERIA AND YEAST UTILIZED AS STARTERS

BY

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A PROJECT WRITTEN AND SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, COLLEGE OF PURE AND APPLIED SCIENCES, CALEB UNIVERSITY, IMOTA, LAGOS STATE, NIGERIA.

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DECLARATION

I, **ZACCHEAUS KANYINSOLA OREOLUWA,** do hereby declare that this project titled "**AMINO ACID PROFILE OF OGI PRODUCED USING LYSINE PRODUCING LACTIC ACID BACTERIA AND YEAST UTILIZED AS STARTERS"** is entirely my work and composition.

The work embodied in this project has not been submitted in any form for any degree or diploma and is not being submitted for any other degree. All references made to works of other persons have been duly acknowledged.

……

Signature/Date

CERTIFICATION

This is to certify that this research project was carried out by ZACCHEAUS KANYINSOLA OREOLUWA with matric number 19/6335 in the Department of Biological Sciences and Biotechnology, Microbiology, Program, College of Pure and Applied Sciences, Caleb University, Imota, Lagos, Nigeria.

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DEDICATION

This work is dedicated to the Almighty God, the giver of wisdom, knowledge and understanding and to my parents Mr and Mrs Zaccheaus for seeing me through my university education.

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ABSTRACT

Ogi is a very common food among Nigerians consumed by infants from the age of 4 months, children, adults, lactating mothers and convalescents. Amino acids are an important part of diets for humans and animals. A lot of the basic amino acids needed in the system cannot be produced by the body and this is why they are considered essential. The main aim of this study is to isolate, characterize and screen for the production of lysine producing lactic acid bacteria and yeast from *ogi* and amino acid profiling of *ogi* produced using lysine producing lactic acid bacteria and yeast. In this study, red sorghum grain (*Sorghum bicolor*) was collected from Alapere/ Ketu market in Lagos state, Nigeria and transported to the laboratory for the preparation of *Ogi*. Ten lactic acid bacteria and ten yeasts were isolated from laboratory prepared sorghum *Ogi* on Malt Extract agar and De Man rogosa sharpe agar and identified on the basis of morphological, physiological and biochemical tests. A total of ten LAB and ten yeast isolates were screened for lysine production on the solid agar medium seeded with lysine auxotroph *Escherichia coli*. Five LAB and five yeasts showed halo growth of the *E. coli* auxotroph on the surface of the medium after incubation, indicating lysine production by the yeast isolates. The lysine produced by the yeast isolates was quantified. The highest lysine producing LAB and yeast *Lactobacillus planterum and Saccharomyces specie* were used as starter culture for the production of *Ogi*, a fermented sorghum gruel. The amino acid profile of the *ogi* which was produced was analyzed. Each sample had glutamic acid as their most abundant amino acid while the least abundant amino acid was between cysteine and methionine. In conclusion, this study shows that the presence of lysine producing lactic acid bacteria and yeast increases the yield of essential amino acids in *Ogi* produced from sorghum.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Ogi is a fermented cereal pudding made from maize, sorghum or millet.

Ogi is a very common food among Nigerians consumed by infants from the age of 4 months, children, adults, lactating mothers and convalescents. According to research, majority of Nigerians consume ogi at least once a week. (Bankole and Omemu 2015).

Lactic acid bacteria (Lactobacillus plantarum and Streptococcus lactis) and yeast (Saccharomyces cerevisiae, Rhodotorula spp., Candida mycoderma and Debaromyces hansenii) are dominantly in the fermentation process of ogi responsible for the enhancement of taste, development of aroma, stability of microbes (Omemu et al, 2011; Aworh, 2008).

Amino acids are an important part of diets for humans and animals. A lot of the basic amino acids needed in the system cannot be produced by the body and this is why they are considered essential.

A lack of any of the amino acids known to be essential can lead to very poor health and in extreme cases death.

For this reason, amino acid profiling is important to determine the quality and quantity of amino acid in food.

Lysine is an essential amino acid that is necessary for human health but cannot be produced by the body.

. Lysine is an important amino acid found in foods such as beef, cheese, eggs, and milk. The amino acid lysine is involved in the formation of collagen, a protein

present in connective tissues and bones such as tendon, skin, and cartilages (Teniola et al., 2001).

The amino acid composition of ogi produced from sorghum shows that the first essential amino acid is lysine, and the second essential amino acid is threonine, the lysine level content meets less than 40% of the recommended level for infants. (Sergio et al 2002)

1.2 STATEMENT OF PROBLEM

A lot of the basic amino acids needed for growth and development of the body cannot be produced by the body and therefore has to be obtained from various food sources, which is why they are considered essential.

1.3 JUSTIFICATION OF STUDY

The purpose of this study is to increase the yield of essential amino acids in ogi produced from sorghum by the use of starter cultures lactic acid bacteria and yeast.

1.4 AIM

To isolate, characterize and screen for the production of lysine producing lactic acid bacteria and yeast from ogi and amino acid profiling of ogi produced using lysine producing lactic acid bacteria and yeast

1.5 OBJECTIVES

To isolate and characterize lysine producing lactic acid bacteria and yeast from ogi

To screen and quantify lysine producing lactic acid bacteria and yeast from ogi

To determine the amino acid profile of ogi produced using lactic acid bacteria and yeast as starter culture.

CHAPTER 2

LITERATURE REVIEW

2.1 OGI

Ogi which is commonly known as pap and is also called *akamu* by the igbos is a Nigerian meal made from fermented corn. It is a fermented cereal pudding usually made from maize, soghurm or millet. (Abosede. and Enujiugha 2018)

The microflora of ogi is mainly dominated by lactic acid bacteria which are generally regarded as safe with *Lactobacillus plantarum* dominating and some specific fungal species which belong to the gnus Aspergillus *niger* (Abosede. and Enujiugha 2018)

Sorghum (*sorghum bicolor)* is the most extensively grown cereal crop in Nigeria, along with maize, it forms a major source of food for people and serves as a source of energy for millions of people in Africa. (Oyaruka and Eleyinmi 2004)

Sorghum comes from the family *poaceae* and has four subspecies; grain soghurm, grass soghurm, guinea corn and broom corn. *Ogi* is produced from grain sorghum. (Apotiola, 2035).

Sorghum contains 9.28% protein, 85.20% carbohydrate, 2.27% fat and 1.27% mineral salts. Sorghum is also a rich source of B-complex vitamins (Apotiola, 2015).

2.2 PRODUCTION OF *OGI*

The common technique of the production of ogi involves fermentation of the grains by soaking in water for about 48hrs before eventually milling into a fine and smooth paste. The slurry is removed and then sieved with a muslin clothe to remove the germ and hull. . (Abosede.and Enujiugha 2020)

The remaining filtrate is then allowed to undergo another round of secondary fermentation for about 24-48 hrs to develop its sour taste. . (Abosede.and Enujiugha 2020)

The length of the secondary fermentation depends on the level of sourness that is required in the ogi. (Abosede.and Enujiugha 2020)

2.3 MICROBIAL PROPERTIES OF OGI

The microbial content of ogi has been seen to be affected by the preparations and ingredients used in the production of *ogi*. (Dozier,2013).

Usually poor sanitary conditions have been seen to affect the food and water where ogi is produced. Some organisms that have been isolated from ogi during preparation are *Aspergillus flavus, Candida albicans, Pseudomonas aeruginosa, Escherichia coli.*

Researchers have found that non enteric bacteria were found in study samples that were screened while the enteric bacteria developed in later stages of fermentation. (Dozier,2013).

Traditional fermentation of ogi is initiated as a result of chanced inoculation by uncontrolled microorganisms from the environment involving a build up of bacteria and fungi.

Different studies have been able to isolate and enumerate possible microorganisms involved in the fermentation of ogi. These are: *Corynebacterium, Candida mycoderma, Fusarium sp, Cephalosporium, Penicillium sp, Rhotdotorula sp, Candida krusei.* . (Salovaara 2004).

Lactic acid bacteria are one of the most common microorganism responsible for cereal fermentation, they are known for their beneficial role towards preservation,

detoxification, enhanced nutritional value, flavor and aroma production. *Lactobacillus plantarum* is reported as the most dominant specie. . (Salovaara 2004)

2.4 NUTRITTIONAL AND CHEMICAL PROPERTIES OF OGI

During fermentation of ogi, some of the organisms involved in fermentation produce amylotytic enzymes which are responsible for the disintegration of the starch substrate to reducing sugars, thereby resulting in the decrease of total sugar content of the ogi.

Lowering of pH is also caused by the ability of the LAB and yeast present in the Ogi during fermentation of the sorghum to utilize the free sugars (Modu et al 2005)

During the fermentation process of producing Ogi, there is usually a significant loss in nutritive value. There are vitamins such as folic acids, niacin, pathogenic acids and thiamine.

Processing such as steeping, milling and sieving result in great reduction in nutrient content (Aminigo and Akingbala,2004).

One of the common practices during the production of ogi is the discarding of the water it has been steeped in during processing. This usually results in the reduction of minerals and other nutrients found in ogi.

In a study on the nutritional quality of Ogi produced using corn, sorghum and millet grains, it was reported that the amino acid composition of ogi produced from sorghum had the most abundance of phenylalanine, glycine, arginine and valine. (Oyarekua and Eleyinmi 2004).

As a result of this, in the choice in weaning food for children, Ogi produced from sorghum would be preferable because of its high value of arginine compared to the quantity in Ogi produced from corn and millet. (Oyarekua and Eleyinmi 2004).

2.5 LACTIC ACID BACTERIA

Lactic acid bacteria (LAB) are gram positive rod/cocci that are aerotolerant, acid tolerant often non-sporulating and non-respiring bacteria that play an important role in the traditional food fermentation process. They aid in the preservation of food by inhibiting the growth of pathogenic organisms like bacteria as well as improving the flavor and texture of food (O'bryan *et al*., 2015).

Lactic acid bacteria (LAB) are common microorganisms that thrive in environments that are high in carbohydrates such as plants, fermented foods, and the mucosal surfaces of people, terrestrial and aquatic animals. (Barinov et al., 2011) (Aureli et al., 2011).

Lactic acid bacteria has been utilized in the preservation of food as well as the altering of organoleptic qualities such as flavor and texture (Barinov,*et al* 2011).

Lactic acid bacteria (Lactobacillus plantarum and Streptococcus lactis) are dominantly in the fermentation process of ogi responsible for the enhancement of taste, development of aroma, stability of microbes (Omemu et al, 2011;)

2.6 YEAST

Yeasts are mostly known for their positive contributions in the fermentation of a lot of products. Studies have shown that yeast is not only important in fermentation of Ogi but in spoilage too (Omemu et al 2007)

The study of the interrelationship between yeast and lactic acid bacteria shows that the growth of yeast strains during fermentation are enhanced by the presence of lactic acid bacteria (omemu et al 2007)

Yeast (Saccharomyces *cerevisiae, Rhodotorula* spp., *Candida mycoderma* and *Debaromyces hansenii*) present in fermentation of Ogi secretes extracellular

enzymes and also possess amylolytic activities which aids in the breakdown of carbohydrates to simple sugars for the use of lactic acid bacteria. (omemu et al 2007)

Presence of yeast in fermentation also helps in preservation,aroma and flavor improvement.(Dahunsi 2019)

2.7 LYSINE

Lysine is an essential amino acid, it is essential in the sense that it cannot be produced by the human body, therefore it must be gotten externally, by the human diet or by supplementation.

Lysine is a building block needed for the biosynthesis of proteins.

Lysine is an important amino acid that is mostly used as a feed additive for animals including broilers, chickens, and pigs, as well as a supplement for people to improve feed quality by enhancing the absorption of other amino acids.

Higher organisms cannot synthesize lysine, which makes it an indispensable amino acid which should be consumed in adequate amounts to maintain protein synthesis. (Dwine E.M 2020).

Lysine is reported to be low in most type of cereals, but sorghum *ogi* has a higher content than other cereals. *Ogi* produced from sorghum is usually supplemented with milk or legumes that are high in lysine. (Modu *et al*, 2007).

2.8 AMINO ACIDS

Amino acids are organic compounds that contain amino (-NH3) and carboxylate (-C02) functional groups along with a side chain specific to each amino acid.

They are often referred to as building blocks of protein and are compouns that play many critical roles in the body. (Jillian 2022)

Amino acids are categorized into essential, conditionally essential and non-essential.

The body needs 20 different amino acids to grow properly. While they are all important for the health. Only 9 are classified as essential. These are: histdine, leucine, lysine, valine, phenylalaline, threonine, methionine, tryptophan and valine.

The body does not make essential amino acids (it is best gotten from a diet) while it can make non-essential amino acids.

Non-essential amino acids include; alanine, asparagine, aspartic acid, cysteine, arginine, glycine, glutamine, serine and tyrosine.

Several non-essential amino acids are classified as conditionally essential, meaning that they are essential only under specific conditions like pregnancy, illness, infancy or trauma (Jillian, 2022).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

De man Rogosa and Sharpe(MRS), conical flask, spatula, autoclave, distilled water; inoculating loop, petri dishes, beaker, electronic weighing balance, test tubes, sterile syringe, aluminum foil, paper tape, spirit lamp, incubator, glass slides, microscope, pH meter, sorghum *Ogi* Bunsen burner, incubator, conical flasks, wash bottle, inoculating loop, cotton wool, alcohol, spatula, measuring cylinder, spectrophotometer, hydrogen peroxide, oxidase strips, glass slides, crystal violet, safranin, decolourizer, iodine, agar , glucose, $(NH_4)_2SO_4$ (ammonium sulphate), $CaCO_3$ (calcium carbonate), K_2HPO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $MnSO_4.H_2O$, peptone, yeast extract, NaCl, $FeSO_4.7H$ O, trichloroacetic acid, phosphate buffer, centrifuge, decanting funnel, muslin clothe, sieve.

3.2 METHODS

COLLECTION OF SAMPLES:

Samples (*ogi* produced from sorghum grains) were purchased from; Festac, Lawanson, Akute, Ketu, Anthony, and Alapere and labeled A-F. The *Ogi* samples were transported to the laboratory for immediate isolation of lysine producing yeast and lactic acid bacteria.

PREPARATION OF CULTURE MEDIA

Method: De man Rogosa and Sharpe Agar was used for the isolation of LAB and was prepared according to the manufacturer's instructions and all media was autoclaved at 121^{o} C for 15 minutes.

Malt extract Agar was used for the isolation of yeast and was prepared according to the manufacturer's instructions and all media was autoclaved at 121^{o} C for 15 minutes

ISOLATION OF LYSINE-PRODUCING YEAST AND LACTIC ACID BACTERIA FROM SORGHUM OGI

Pour plate technique was used to isolate yeast and lactic acid from sorghum *Ogi*. In washed test tubes, 9ml of distilled water was poured, autoclaved for 15 minutes at 121°C, and allowed to cool. 1g each of sorghum *Ogi* from samples A-F were aseptically transferred into 9ml of sterile distilled water in a test tube and shaken thoroughly for 2min. 1ml of the stock solution was serially diluted up to fivefold (10^{-5}). 10^{-3} and 10^{-5} fold serial dilution was inoculated using pour plate method for yeast on PDA and MEA and then incubated for 4-5 days at 25-30^{o} C.

The 10^{-3} and 10^{-5}fold serial dilution was inoculated into the MRS medium to isolate lactic acid bacteria. The plates were in the incubator for a period of 48 hours 25-30^{o} C

After incubation, colonies that developed on the plates were examined for the development of distinct colonies. The colonies were counted and randomly selected and sub-cultured by streaking onto a fresh PDA plate to obtain a pure culture of Yeast isolate at 25-30^{o} C for 4-5 days.

SCREENING FOR LYSINE PRODUCING YEAST ISOLATED FROM *OGI*

The isolates were tested/ screened for lysine synthesis using a minimum medium containing glucose, 4.0g; $(NH_4)2SO_4$, 2.0; K_2HPO_4, 0.5g; KH_2PO_4, 0.5;

$MgSO_4.7H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $MnSO_4.4H_2O$, 0.00lg; $CaCO_3$, 2g; Agar, 15.0g; and water, 1 litre of water with the pH adjusted to 6.0 (Ozulu U. S., 2012). The isolates were inoculated into a medium that had been seeded with a 24 hour broth culture of the lysine auxotroph, *Escherichia coli* (NCCB 1841). As a control, an uninoculated agar plate was used. The plates were evaluated for auxotroph growth after 96 hours of incubation at 30° C. Halo growth of the *E. coli* indicates lysine production by the isolate (Okpalla J. *et al*., 2019).

SCREENING FOR LYSINE PRODUING LACTIC ACID BACTERIA ISOLATED FROM *OGI*

The isolates was evaluated for lysine production using a minimal medium containing glucose, 4.0g; $(NH_4)_2SO_4$ (ammonium sulphate), 2.0g; $CaCO_3$ (calcium carbonate), 2g; KH_2PO_4, 0.5g; K_2HPO_4, 0.5g; $MgSO_4.7H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $MnSO_4.H_2O$, 0.001g; agar, 15.0g; and water, 1 litre of the medium with the pH adjusted to the isolate was inoculated into a medium containing a 24 hour broth culture of *Escherichia coli*, a lysine auxotroph (NCCB 1841). An agar plate that has not been inoculated was used as a control. After seventy two hours of incubation at 30ºC, the plates were examined for auxotroph growth. (Okpalla et al, 2019).

Quantification of Lysine Produced Lysine-Producing Yeast and LAB Isolated from *Ogi*, a Fermented Sorghum Gruel

Ninhydrin technique was used to determine the amount of lysine in the broth culture. A 24 h yeast culture broth was centrifuged at 5000 xg for 20 minutes, the cell-free supernatant was collected and lysine production was measured. In a test tube, 1 ml glacial acetic acid was added into 1 ml of the cell-free supernatant. The supernatant in the test tube was then dispensed into 1 ml of a reagent solution containing an acid combination; (0.4 ml of 6M orthophosphoric acid, 0.6 ml of glacial acetic acid, and 25 mg of ninhydrin. 1 ml glacial acetic acid, 1 ml acid mixture without ninhydrin,

and 1ml supernatant to make up a blank). The tubes were sealed, and the contents were homogenized for 10 minutes before being heated in a water bath at 100°C for 1 h. The test tubes were quickly cooled under running water, and 2 ml of glacial acetic acid was dispensed into each test tube to make a total volume of 5 ml. Using a spectrophotometer, the optical density of the reacting mixture was measured at 515 nm against a blank. A standard lysine curve was used to extrapolate the test samples' results (Ekwealor *et al.*, 2005).

PRODUCTION OF OGI FROM SORGHUM

Sorghum grains were purchased from, Alapere, Ketu, the grains were washed thoroughly to remove dirt.

The grains were soaked in room tempterature water for 72 hours before it was milled into a fine paste.

. It was removed and then sieved with a muslin clothe to remove the germ and hull.

The remaining filtrate was then allowed to undergo another round of secondary fermentation for about 24-48 hrs to develop its sour taste.

EXRACTION AND EVALUATION OF AMINO ACID FROM *OGI* PRODUCED WITH LYSINE PRODUCING LAB AND YEAST.

2.0 g of ogi samples were weighed using a digital chemical balance and was transferred into 20ml of 0.2M phosphate buffer solution at 7.0 PH.

The mixture was centrifuged at 2000rpm for 10min, the supernatant was decanted and poured into a separating funnel.

The supernatant was shaken three times with 10ml petroleum ether to remove the organic pigments. The top phase was discarded and the aqueous phase which contained protein and amino acids was retained

Protein was precipitated out from the aqueous phase by adding 5.0ml of 10% trichloroacetic acid to 5.0ml extract. The mixture was shaken and kept in the freezer for 10mins. The precipitate formed was removed by centrifugation and the filtrate was used for the amino acid profile determination.

CHAPTER 4

RESULTS

Ten LAB and ten yeast were isolated from laboratory made sorghum *Ogi* and identified on the basis of morphological tests; microscopic and macroscopic examination, physiological tests and biochemical tests and were identified according to Mycology manual for yeast and Berges manual for LAB.

The LAB species identified are

Lactobacillus planterum, Lactobacillus curvatus, Lactobacillus delbruecki, Lactobacillus audophillus and Lactobacillus casei.

The LAB isolates exhibited morphological characteristics with colors cream with small, tiny colonies in the shape of rods and short rods on the De Man, Rogosa and Sharpe agar plates. (Table 4.1)

The physiological tests carried out showed that a variety of the LAB isolates grew at temperatures of 15^{o}C and 45^{o}C respectively, they all grew at pH concentrations of 3.9 and 9.6. A variety of the LAB isolates were able to hydrolyze starch. The biochemical tests showed that the LAB isolates had variation in the fermentation of glucose, maltose, fructose, sucrose. All LAB isolates showed negative reactions to motility test and all LAB isolates showed negative reactions to catalase test. All the LAB isolates revealed a negative reaction to the oxidase and indole test. (Table 4.1)

The yeasts identified were *Saccharomyces specie, Candida specie, Candida parapsilosis and Saccharomyces cerevisiae.* With *Candida specie* as the highest occurring. (Table 4.2)

The yeast isolates exhibited morphological characteristics with color ranging from cream to white with flat, smooth, irregular, raised, undulating, glossy, opaque, small, medium colonies on the Malt Extract Agar plates. (Table 4.2)

The physiological tests carried out showed that a variety of the yeast isolates grew at temperatures of 15ºC and 45ºC respectively, they all grew at pH concentrations of 2.5 and 3.9. A variety of the yeast isolates were able to hydrolyze starch and assimilate Potassium nitrate (KNO_3). (Table 4.2)

The biochemical tests showed that the yeast isolates had variation in the fermentation of glucose, maltose, fructose, mannitol, sucrose, galactose, lactose and xylose. All yeast isolates showed negative reactions to motility test and all yeast isolates showed positive reactions to catalase test. All the yeast isolates revealed a negative reaction to the oxidase and indole test with the exception of a few yeast isolates that showed a positive oxidase test. (Table 4.2)

A total of 10 LAB and 10 yeasts were screened for lysine production. 5 LAB and 5 yeast showed positive result to lysine screening (Table 4.3 and 4.4)

The lysine produce LAB and yeast were quantified in a submerged medium using a spectrophotometer. (Table 4.5 and 4.6)

The highest producing LAB and yeast which were *Lactobacillus planterum* and *Saccharomyces specie* were used as starter cultures in the production of *ogi.*

There were four samples of *ogi* produced with sample A inoculated with *Lactobacillus planterum* sample B inoculated with *Saccharomyces specie,* sample C inoculated with a combination of both the LAB and yeast and sample D was used as control.

The amino acid profiles were carried out on these four samples which showed that sample C which was inoculated with a combination of both LAB and yeast had the highest abundance of amino acids. The amino acid with the most abundance were

glutamic acid and sermine while the amino acids with the least abundance were methionine and cysteine. (table 4.7)

Table 4.1: Morphological, Biochemical And Physiological Characteristics Of Lactic Acid Bacteria Isolated From *Ogi*

Isolate Code	Colony Morphology	Gram Stain	Reaction	Cellular Morphology	Growth At 15°C	45°C	Ph3.9	Ph9.6	Casein	Starch	Catalase	Oxidase	Motility	Indole	MR	VP	Glucose	Maltose	Sucrose	NH3 from	fructose	Probable Identity
LBO1	Small-sized, creamy colony	+		Rods	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	+	*Lactobacillus planterum*
LB02	Creamy tiny colony	+		Short rods	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	+	*Lactobacillus curvatus*
LB03	Small-sized, creamy colony	+		Rods	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	+	*Lactobacillus planterum*
LB04	Creamy tiny colony	+		Short rods	-	+	+	+	+	+	-	-	-	-	+	-	+	-	-	+	+	*Lactobacillus delbruecki*
LB05	Creamy tiny colony	+		Short rods	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	+	*Lactobacillus planterum*

LB06	Small sized creamy colony	+	rods	-	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	*Lactobacillus corvatus*
LB07	Creamy tiny colony	+	Short rods	-	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	+	*Lactobacillus audophillus*
LB08	Small-sized, creamy colony	+	Short rods	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	+	*Lactobacillus casei*
LB09	Creamy tiny colony	+	Rods	+	-	+	-	+	+	-	-	-	-	+	-	+	+	+	+	-	*Lactobacillus buchneri*
LB10	Small-sized, creamy colony	+	Rods	+	+	+	+	+	+	-	-	-	-	+	-	+	-	+	+	+	*Lactobacillus fermentum*

Table 4. 2: Morphological, Biochemical and Physiological Characteristics Of Yeast Isolated From *Ogi*

Isolate code	Colony morphology	Cellular morphology	Growth at	45 °c	pH3.9	pH9.6	KNO3	Starch	Catalase	Oxidase	motility	indole	glucose	maltose	fructose	mannitol	sucrose	galactose	lactose	Probable identity
Y01	Creamy, flat, dull, small-sized, opaque colony	Oval, shaped elongated cells	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+	+	-	*Saccharomyces specie*
Y02	Creamy, round, small sized, smooth colony	Small, oval shaped cells	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	*Candida specie*
Y03	Creamy, round, small sized, smooth colony	Small, oval shaped cells	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	*Candida specie*

Y04	Creamy, flat, dull, small-sized, opaque colony	Oval, shaped elongated cells	+	+	+	+		-	+	-	+	-	+	+	+	+	+	+	-	*Saccharomyces specie*
Y05	Creamy, round, small sized, smooth colony	Small, oval shaped cells	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	*Candida specie*
Y06	Creamy, round, small sized, smooth colony	Small, oval shaped cells	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	*Candida specie*
Y07	Creamy, flat, round, smooth, raised, glossy, opaque colony	Small cluster, oval shaped cells	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	*Candida parapsilosis*

Y08	Creamy, medium sized, round, flat, smooth, raised, undulating, colony	Small cluster, oval shaped cells	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	*Saccharomyces cerevisiae*
Y09	Creamy, round, small sized, smooth colony	Small, oval shaped cells	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	*Candida specie*
Y10	Creamy, round, flat, smooth, raised, glossy, opaque colony	Small cluster, oval shaped cells	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	*Candida parapsilosis*

Table 4.3: Screening of LAB isolated from *Ogi* for lysine production

Probable LAB isolate	Halo growth of *Escherichia coli*
lactobacillus plantarum	+
lactobacillus curvatus	+
lactobacillus plantarum	+
lactobacillus delbruecki	+
lactobacillus plantarum	+
lactobacillus curvatus	-
lactobacillus audophillus	-
lactobacillus casei	-
lactobacillus buchneri	-

Table 4.4: Screening of yeast isolated from *Ogi* for lysine production

Probable yeast isolates	Halo growth of *Escherichia coli*
Saccharomyces species	+
Candida species	+
Candida species	+
Saccharomyces species	+
Candida species	+
Candida species	+
Candida parapsilosis	-
Saccharomyces cerevisiae	-
Candida species	-
Candida parapsilosis	-

Table 4.5: Quantification of Lysine Produced in Lyine Producing LAB isolated From *Ogi*

LAB isolate	Lysine quantity (mg/ 100g)
lactobacillus plantarum	4231.43
lactobacillus curvatus	1757.14
lactobacillus plantarum	1184.29
lactobacillus delbruecki	686.43
lactobacillus plantarum	945.71

Table 4.6: Quantification of Lysine Produced in Lyine Producing yeast isolated From *Ogi*

Yeast isolate	Lysine quantity (mg/ 100g)
Saccharomyces species	819.28
Candida species	510.00
Candida species	434.28
Saccharomyces species	412.86
Candida species	189.28

Table 4.7: Amino Acid Profile of *Ogi* Produced Using Lab And Yeast As Starter Culture

	STARTER CULTURE							
	(*lactobacillus plantarum)* (mg/ 100g)		(*Saccharomyces* specie) (mg/ 100g)		(*lactobacillus plantarum*+ *Saccharomyces* specie) (mg/ 100g)		(control) (mg/ 100g)	
Amino Acids								
Lysine	342.86	350.00	321.43	314.28	378.57	378.57	278.57	271.43
Leucine	600.00	592.86	728.57	735.71	764.29	771.43	528.57	514.29
Threonine	407.14	407.14	485.71	485.71	535.71	542.86	378.57	392.86
Valine	535.71	542.86	550.00	557.14	571.43	571.43	457.14	457.14
Iso-Leucine	864.29	864.29	921.43	914.29	942.86	928.56	728.57	728.57
Phenylalanine	371.43	364.29	400.00	407.14	428.57	428.57	321.43	328.57

Cysteine	192.86	192.86	200.00	207.14	221.43	228.57	171.43	164.29
Thyrosine	300.00	300.00	357.14	357.14	392.86	392.86	285.71	285.71
Alanine	292.86	292.86	314.29	314.29	350.00	350.00	264.29	264.29
Glycine	335.71	342.86	350.00	350.00	371.43	364.29	300.00	300.00
Glutamic Acid	1157.14	1157.14	1178.57	1178.57	1192.86	1192.86	1107.14	1114.29
Proline	342.86	342.86	364.29	364.29	378.57	378.57	314.28	314.28
Sermine	1100.00	1100.00	1114.29	1114.29	1121.43	1121.43	1057.14	1057.14
Arginine	835.71	835.71	864.29	871.43	892.86	900.00	778.57	778.57
Aspartic Acid	764.29	757.14	778.57	785.71	821.43	814.29	707.14	707.14
Histidine	364.28.	364.28	414.28	414.28	400.00	407.14	328.57	314.28
Methionine	164.28	164.28	178.57	178.57	192.59	192.59	142.86	142.86

STATISTICAL ANALYSIS OF AMINO ACID PROFILE OF *OGI* PRODUCED USING LAB AND YEAST AS STARTER CULTURE

CULTURE	LYSINE	LEUCINE	THREONINE	VALINE	ISOLEUCINE	PHENYLALANINE
A	346.43±5.05^{b}	596.43±5.05^{b}	407.14±0.00^{ab}	539.29±5.06^{b}	864.29±0.00^{b}	367.86±5.05^{b}
B	317.86±5.06^{a}	732.14±5.05^{c}	485.71±0.00^{c}	553.57±5.05^{c}	917.86±0.00^{c}	403.57±5.05^{c}
C	378.57±0.00^{c}	767.86±5.05^{d}	539.29±5.06^{d}	571.43±0.00^{d}	935.71±0.00^{c}	428.57±0.00^{d}
D	378.57±0.00^{c}	521.43±10.10^{a}	385.72±10.11^{a}	457.14±0.00^{a}	728.57±0.00^{a}	325.00±5.05^{a}

CULTURE	CYSTEINE	THYROSINE	ALANINE	GLYCINE	GLUTAMIC ACID	PROLINE
A	192.86±0.00^{b}	300.00±0.00^{b}	292.86±0.00^{b}	339.29±5.06^{b}	1157.14±0.00^{b}	342.86±0.00^{b}
B	203.57±5.05^{b}	357.14±0.00^{c}	314.29±0.00^{c}	350.00±0.00^{c}	1178.57±0.00^{c}	364.29±0.00^{c}
C	225.00±5.05^{c}	392.86±0.00^{d}	350.00±0.00^{d}	367.86±5.05^{cd}	1192.86±0.00^{d}	378.57±0.00^{d}
D	167.86±5.05^{a}	285.71±0.00^{a}	264.29±0.00^{a}	300.00±0.00^{a}	1110.72±5.06^{a}	314.28±0.00^{a}

CULTURE	SERINE	ARGININE	ASPARTIC ACID	HISTIDINE	METHIONINE
A	1100.00±0.00^{b}	835.71±0.00^{b}	760.72±5.06^{b}	364.28±0.00^{b}	164.28±0.00^{b}
B	1114.29±0.00^{c}	867.86±5.05^{c}	782.14±5.05^{c}	414.28±0.00^{c}	178.57±0.00^{c}
C	1121.43±0.00^{d}	896.43±5.05^{d}	817.86±5.05^{d}	403.57±5.05^{c}	192.59±0.00^{d}
D	1057.14±0.00^{a}	778.57±0.00^{a}	707.14±0.00^{a}	321.43±10.11^{a}	142.86±0.00^{a}

CHAPTER 5

DISCUSSION

In this study, a total of 10 species of Lactic acid bacteria and 10 species of yeast were isolated from *ogi* samples. Generally, in sorghum, lactic acid bacteria is usually the most dominant during fermentation, with lesser occurrence of yeast (Adebo *et al* 2018).

The LAB and yeast isolates were identified based on their morphological, physiological and biochemical test.

The highest occurring percentage of yeast was *Candida specie* while the highest occurring percentage of LAB was *Lactobacillus planterum* which agrees with the findings of Omemu et al, 2011 who said that *Lactobacillus planterum and Candida specie* is predominantly involved in the fermentation of *OGI.*

The LAB species identified were *Lactobacillus planterum, Lactobacillus curvatus, Lactobacillus delbruecki, Lactobacillus audophillus and Lactobacillus casei.*

The yeasts identified were *Saccharomyces specie, Candida specie, Candida parapsilosis and Saccharomyces cerevisiae.*

These LAB and yeast samples were screened for lysine using a minimal medium, 5 of the LAB and 5 of the yeast were seen to be lysine producing.

This agreed with the findings of Odunfa *et al* (2001).

Escherichia coli auxotroph being a mutant organism, requires additional nutrients to grow and thrive, yeast isolates that were able to release lysine onto the agar medium stimulated halo growth of the E. coli auxotroph seeded on the agar. This observation is similar to the investigation by Ozulu et al. (2012) who reported on methionine-producing bacteria. It was observed that only bacterial isolates that released methionine into the agar medium stimulated halo growth of the E. coli auxotroph, seeded on the agar, methionine being an additional nutrient stimulated the halo growth of E. coli auxotroph.

The active lysine producers were studied for the amount of lysine produced and were quantified in a submerged medium.

With the organisms highest producing lysine being *Lactobacillus planterum and Saccharomyces specie.* These samples were then inoculated into traditionally made sorghum based *ogi.*

The results of the quantification shows that LAB produced more lysine than yeast

4 samples of *ogi* were analyzed, with each sample containing LAB *Lactobacillus plantarum*, yeast *saccharomyces species*, LAB and yeast together and one final sample which was used as control.

The samples were analyzed for lysine, threonine, valine, iso-leucine, phenylalanine, cysaline, tyrosine, alanine, glycine, glutamic acid, roline, sermine, arginine, aspartic acid, histidine, and methionine according to the method of

Each sample had glutamic acid as their most abundant amino acid while the least abundant amino acid was between cysteine and methionine.

. Lysine-producing yeasts and LAB present in Ogi fermentation process have revealed to bring about the increase in Ogi's nutritional value thereby increasing the amount of lysine in the body when Ogi is consumed. (Ekwealor and Orafu, 2003). Adisa et al. (2019) stated that lysine-producing yeasts help in the fermentation process of food, they make the fermented product appealing, attractive and also increase its sensory properties and health advantages.

The presence of lysine-producing lactic acid bacteria and yeast in food is shown to increase the yield of essential amino acids which can’t regularly be produced by the human body.

CONCLUSION

In conclusion, the study has clearly revealed that lysine-producing yeast and LAB can be isolated from *Ogi*, a fermented sorghum gruel and can be screened and quantified for lysine production.

These isolated organisms can be used to improve the amino acid content of *Ogi*, a fermented sorghum gruel and provide more sources of essential amino acids which is needed by the body but cannot be produced by the human system.

This research contributes to the usefulness of LAB and yeast as starter culture to increase the nutritional value of fermented foods.

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