Original Research Article

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF LACTIC ACID BACTERIA AND YEASTS INVOLVED IN SPONTANEOUS FERMENTATION OF '*OGI*', A CEREAL-PORRIDGE PRODUCED IN NIGERIA

Abstract

The traditional fermented cereal-porridge 'ogi' is an important dietary staple food popularly consumed in Nigeria. The fermentation is mainly driven by lactic acid bacteria and yeasts during spontaneous fermentation. This study aimed to isolate and ascertain the phenotypic and genotypic characteristics of lactic acid bacteria and yeasts associated with spontaneous fermentation of maize and sorghum. Lactic acid bacteria (LAB) and yeasts were isolated from fermented ógi' by spread plate method. A total of 9 isolates of lactic acid bacteria and 4 yeast isolates were obtained and were characterized based on general morphology, Gram-staining and biochemical tests. The lactic acid bacteria identified by molecular method were strains of *Lactobacillus fermentum*, *Enterobacter* spp, and *Lactococcus* spp, while the yeasts were strains of *Trichosporon asahii*, *Hypopichia burtoni* and *Pichia kudriavzevii*.

Keywords: Lactic acid bacteria, yeast, spontaneous fermentation, ogi

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1. Introduction

Spontaneous fermentation is an uncontrolled fermentation dating way back in time, as a traditional method of food preparation and the form of fermentation in most small scale fermentations in the developing countries [1]. The traditional cereal-porridge 'ogi' is produced by spontaneous fermentation of cereals such as maize, sorghum and millet. Several genera of microorganisms are known to associated with the fermentation of steeped maize and sorghum for '*ogi'* production, such as *Staphylococcus*, *Escherichia, Pseudomonas, Enterococcus, Klebsiella, Bacillus, Lactobacilli, Leuconostoc, Clostridium, Corynebacterium, Streptococcus, Micrococcus*

and *Citrobacter* (bacteria), *Aspergillus, Saccharomyces, Penicillin, Candida, Rhizopus, Fusarium, Mucor, Geotrichum*, *Pichia* and *Rhodotorula* (fungi) [1,2-5].

Lactic acid bacteria mainly *Lactobacillus plantarum* and *Lactobacillus fermentum*, and the yeasts *Saccharomyces cerevisiae* and *Pichia kudriavzevii* are the predominant microbial species in fermentation of cereals for the production of 'ogi' [3,5,6]. Majority of the microbes in fermented steeped maize for *ogi* production have been variously reported to participate in fermentation of some other local food [7,8]. These microorganisms are present in the environment and can inoculate suitable substrates at will.

The major function of LAB is to produce lactic acid that is the acidification of the food while its main application is as starter cultures in the food industry with a variety of fermented diary products, meat, fish, vegetable and cereal products. It contributes to the flavour, texture and nutritional value of the fermented foods through production of aroma components and use [9-11]. As probiotics, they confer health benefits upon consumption and inhibit proliferation of spoilage and pathogenic microorganisms [11-13]. Together with yeast, LAB are usually the most commonly used microorganisms for the fermentation of foods [3].

'Ogi' has been produced for a long time by the traditional fermentation technique, which involves a spontaneous and uncontrolled process, usually on small scale basis. A major step in the production of 'ogi' on a large scale would require the isolation and characterization of the microorganism involved in the fermentative process for use as starter cultures. The significance of this study is that it will provide a comparative and accurate information on the culturally(phenotypically) and genotypically identified lactic acid bacteria and yeasts involved in the 'ogi' fermentation process. This will be useful to researchers and industrialists in the selection of starter cultures for controlled, large scale production and industrial processes

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2. Materials and Methods

2.1. Processing of Maize and Sorghum into "Ogi"

Maize and sorghum grains were separately sorted, cleaned and processed using a modified wet-milling laboratory method developed and reported by Ojokoh [14]. The paste was sieved and allowed to sediment and ferment for 48h. The sedimented paste formed the sample for 'ogi' production. The paste is usually used to produce 'ogi' by mixing a measured amount in cold water before boiling or adding hot water to form a thick gel or porridge.

2.2. Microbiological Analysis

2.2.1 Isolation of Lactic Acid Bacteria and Yeast

Five grams (5g) of "Ogi" paste was mixed by swirling in 45ml sterile 0.1% (w/v) peptone water (Oxoid, England) in a conical flask. Tenfold serial dilutions of the mixture of "Ogi" paste in peptone water were made using 0.1% (w/v) peptone water in test tubes and 0.1ml of appropriate dilutions were inoculated in duplicates by spread plate technique on de Man, Rogosa and Sharpe's (MRS: Oxoid, England) agar, for the growth of lactic acid bacteria. The plates were incubated anaerobically in Gallenkamp anaerobic jars for 48 hours. The anaerobic environment was created by the addition of anaerobic gas pack (Thermo SCIENTIFIC, Oxoid Ltd, Basingstoke) to the jars, according to manufacturer's instruction.

Potato Dextrose agar (PDA: OXOID, England) was used for growth of the yeasts. The medium was acidified by the addition of lactic acid (0.1%, v/v) when it was cooled after autoclaving and before pouring to eliminate bacteria. The plates were incubated at 30°C for 72h.

2.2.2 Characterization of Lactic Acid Bacteria

The method of Onwuakor *et al.* [15] was adopted. The parameters for characterization included cultural characteristics, cell morphology, Gram reaction, tests for catalase, indole, oxidase and motility. Other tests included growths in different concentrations of NaCL, different pH

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environments and temperatures and also the fermentation of carbohydrates (D-glucose, ribose, sucrose, lactose, maltose, mannitol, galactose, salicin, sorbitol, mannose, starch and trehalose) (MERCK, Genermany).

2.2.3 Growth of LAB in different concentrations of sodium chloride (NaCl)

De Man, Rogosa and sharpe's agar (MRS: OXOID, England) was prepared according to the manufacturer's information and sterilized by autoclaving at 121°C for 15 min and cooled. It was then inoculated with the test bacteria by pour method after adding 1ml amounts of different concentrations of sterile NaCl to different plates in duplicates. The different concentrations used were 2%, 3%, 4%, 6.5%, 10%. The plates were then incubated anaerobically at 37% for 48h. Growth on the plates indicated a positive result.

2.2.4 Growth of LAB in different pH environment

The MRS agar medium was prepared in 5 different conical flasks. Then the pH of the medium in each of 4 conical flasks was differently adjusted to 4.4, 7.2, 9.3, 9.6 respectively, using the pH meter, with acetate and phosphate buffers. The 5th flask contained the medium with the original pH (6.5), and was used as control. The media were then sterilized and inoculated with the test organisms by pour-plate method and incubated anaerobically for 48h at 37°C. Growth on the plates indicated positive test results.

2.2.5 Growth of LAB at different temperatures

The test bacteria were inoculated by spread-plate method and incubated anaerobically for 48h at different temperatures of 30°C, 40°C, and 45%. Growth on the plates indicated a positive result.

2.2.6 Fermentation of Carbohydrates by LAB

The test medium (MRS broth, Oxoid England) was prepared by mixing 95ml peptone water, and 5ml of 0.2% phenol red (indicator) in a beaker and dispensing 4.75ml volumes into bijou bottles and sterilizing by autoclaving at 121°C for 15min. After cooling, 0.25ml volumes of the filtered test sugar solution was aseptically added to each bottle and mixed to form the test medium. Then

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the sugar test medium was inoculated with a speck of the test bacterium from an isolated colony. The bottles were then incubated anaerobically at 34°C for 48h. A colour change from pale yellow to pink indicated a positive result. The carbohydrates used for testing for viable aerobic yeasts were lactose, sucrose, mannitol, mattose, galactose, fructose, D-Glucose, Ribose, raffinose, arabinose, rhaminose, xylose, mannitol, salicin, sorbitol, mannose, starch and trehalose (MERCK, Germany). A colour change from pale yellow to pink indicated a positive result.

2.2.7 Determination of lactic acid (%)

The method of Aneja [16]was adopted. The LAB were first sub-cultured on MRS Agar and incubated at 37°C under anaerobic conditions for 48h.

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2.2.8 Ethanol Tolerance Test for Yeasts

Potato Dextrose Agar (PDA) (Oxoid, England) was prepared according to the manufacturer's instruction, by dissolving it in distilled water in a conical flask. Then 5ml amounts were dispensed into bijou bottles and sterilized by autoclaving at 121°C for 15min. After cooling to 50°C (cool enough to touch), 1ml of 2%, 4% and 6% ethanol were added in duplicates to different bijou bottles and poured into sterile Petri dishes and left to solidify. Each plate was then inoculated with the test organism by streaking a loopful of 24h broth culture grown on Yeast Extract peptone Dextrose broth (MERCK, Germany), per plate in duplicates. A positive result was the growth of the test organism on the medium.

2.3 Molecular Identification Of Lactic Acid Bacteria And Yeasts

2.3.1 Bacterial Genomic DNA Extraction

The physical or boiling method of Okolie et al. [17] was adopted. One isolated colony of each test bacterium was inoculated in Luria Bertani broth (LB: Miller, Amresco) in bijou bottles and incubated anaerobically for 12h. Then 1ml of the broth culture was transferred to Eppendorf tube

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(microcentrifuge tube) followed by the addition of 0.5ml normal saline and centrifuged at 12,000 rpm for 5min, using Eppendorf centrifuge (5424), to wash the cells. The supernatant was decanted and 1ml of normal saline was added to the sediment and homogenized for 5min using the votex mixer (Celtech XH-B) and centrifuged again. The washing process was repeated 2 more times with the sediment. Then 0.5ml DNA elution buffer (Zymo Research, USA) was added to the resultant sediment and homogenized using the votex and heated with the heating block (Wealtec HB-2 USA) at 95°C for 20min. The heated bacterial suspension was cooled in the deep freezer at -20°C for 10min and spun for 3min at 14,000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20°C for other downstream reactions.

2.3.2 Bacterial DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer (Thermos scientific).

2.3.3 Bacterial 16S rRNA Amplification

The 16S rRNA of the rRNA genes of the isolates were amplified using the

27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3'

primers on an ABI 9700 Applied Biosystems thermal cycler (Dyad) at a final volume of 50µl for 35 cycles. The PCR mix included: the X2 dream taq Master mix supplied by Inqaba, South Africa (taq polymerase. DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5min; denaturation, 95°C for 30sec; annealing, 52°C for 30sec; extension, 72°C for 30sec for 35 cycle and final extension, 72°C for 5min. The product was resolved on a 1% agarose gel (Inqaba, South Africa) at 120V for 15min and visualized on a UV transilluminator (Prio-surplus).

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2.3.4 Fungal genomic DNA Extraction

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa following the manufacturer's protocol. A heavy growth of the pure culture of the fungal isolates was suspended in 200µl of isotonic buffer in a ZR Bashing Bead Lysis tube, then 750µl of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5min. The ZR bashing bead lysis tubes were centrifuged at 10,000 × g for 1 minute.

Four hundred (400) microlitres of supernatant was transferred to a Zymo-Spin IV spin filter (Orange top) in a collection tube and centrifuged at 7000 × g for 1minute. One thousand two hundred (1,200) microlitres of fungal/bacterial DNA binding buffer was added to the filtrate in the collection tubes, bringing the final volume to 1600µl. Then 800µl was transferred to a Zymo-spin IIC column in a collection tube and centrifuged at 10,000 × g for 1min and the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliters of the DNA pre-wash buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000 × g for 1min followed by the addition of 500µl of fungal/bacterial DNA wash buffer and centrifuged at 10,000xg for 1min.

The Zymo-spin IIC Colum was transferred to a clean 1.5µl centrifuge tube, 100µl of DNA elution buffer was added to the Colum matrix and centrifuged at 10,000 × g for 30sec to elute the DNA. The ultra pure DNA was then stored at -20 degree for other downstream reactions.

2.3.5 Internal transcribed Spacer (ITS) Amplification of Yeast DNA

The ITS region of the rRNA genes of the isolate were amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4R: 5'-TCCTCCGCTTATTGATATGC-3', primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50µl for 35 cycles. The PCR mix included: the X2 Dream tag Master mix supplied by Inqaba, South Africa

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(taq polymerase, DNATPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5min denaturatoin, and 95°C for 30sec; annealing, 53°C for 30sec; extension, 72°C for 30sec for 35 cycles and final extension, 72°C for 5min.

2.3.6 Sequencing

Sequencing was done using the BigDye terminator kit on a 3510 ABI sequencer by Inqaba, South Africa. The sequencing was done at a final volume of 10µl, the components included 0.25µl BigDye$^{(R)}$terminator vl.1/v.3.1, 2.25µl of 5 x BigDye sequencing buffer, 10µl PCR Primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10s, 55°C for 5sec and 60°C for 4min.

2.3.8 Phylogentic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded from the National Centre for Biotechnology information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-joining method in MECA 6.0 [18]. The bootstrap consensus tree inferred from 500 replicates [19] is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [20].

3. Results

3.1 Phenotypic Characteristics of Isolates

Table 1 shows the phenotypic characteristics of lactic acid bacterial isolates from the 'ogi' samples. Table 1 shows the characteristics of the yeast isolates.

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Table 1: Phenotypic Characteristics of lactic acid bacterial isolates

Isolate Code	Gram reaction	Cell Morphology	oxidase	catalsase	Motility	D-Glucose	Ribose	Sucrose	Lactose	Maltose	Fructose	Raffinose	Arabinose	Rhaminose	Xylose	Mannitol	Galactose	Salicin	Sorbitol	Mannose	Starch	Trehalose	Indole	Nitrate red.	Nacl (%)					pH				Temp.			Lactic acid (%)
																									2	3	4	6.5	10	4.4	7.2	9.3	9.6	30°C	40°C	45°C	
B1	+ve	Cocci	-	-	-	A/G	A/G	A/G	A/G	A	A	A/G	A	A	-	A	A/G	A/G	A	A	-	A/G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1.22
B2	+ve	Cocci	-	-	-	A	A	A	A	A	A	-	-	A	A	-	+	-	-	A	+	+	-	-	+	+	+	+	-	+	+	-	-	+	-	-	0.58
B3	+ve	Cocci	-	-	-	A	A	A	-	A	A	-	A	-	-	-	A	A	-	A	-	A	-	+	+	+	+	+	-	+	+	+	+	+	+	+	0.27
B4	+ ve	Cocci	+	+	+	A	A	A	A	A	A	A	A	-	A	-	A	A	-	-	-	A	-	-	+	+	+	+	+	-	+	+	+	+	+	-	0.60
B5	+ ve	Cocci	-	-	-	A	A	A	-	A	A	-	A	-	-	-	A	A	-	A	-	A	-	+	+	+	+	+	-	+	+	+	+	+	+	+	1.54
B6	+ ve	Cocci	-	-	-	A	A	A	A	A	A	A	A	A	-	A	-	-	A	A	A	A	-	-	+	+	+	+	-	+	+	-	-	+	+	+	0.95
B7	+ ve	Cocci	-	+	-	A	A	A	A	A	A	-	-	-	-	A	A	A	A	A	-	A	-	-	+	+	+	+	-	+	+	-	-	+	+	-	1.24
B8	+ ve	Cocci	-	-	+	A	A	A	A	A	A	A	A	A	A	A	A	-	-	A	-	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	0.45
B9	+ ve	Cocci	-	-	+	A	A	A	-	A	A	A	A	-	A	A	A	A	A	A	+	A	-	-	+	+	+	+	-	+	+	+	-	+	+	-	0.28

Table 2: Characteristics of yeast isolates

Isolate Code	Cultural Characteristics	Gram Stain	Shape	Sugar Fermentation						Ethanol tolerance
				Glucose	Maltose	Galactose	Sucrose	Lactose	Fructose	
Y1	2mm, mucoid, milky, rough edges	+	Oval	+	+	-	+	-	+	+
Y2	1mm, yellow round edge	+	Oval	+	+	-	-	-	+	+
Y3	3mm, dry surface, pointed centre, smooth edges	+	Oval	+	+	-	+	-	-	-
Y4	1mm pale milky round, smooth edges	+	Oval	+	+	-	+	-	+	+

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3.2 Result of the 16S rRNA Amplification of bacterial isolates on the Agarose gel electrophoresis of the 16S rRNA

The result is shown in Plate 1. Lanes 1-6 represent the 16S rRNA bands at 1,500bp while lane l represents the 100bp molecular ladder.

3.3 Result of ITS (Internal Transcribed Spacer) Amplification of yeast DNA

The PCR result is shown on the agarose gel electrophoresis showing the amplified ITS of the fungal isolates (Plate 2). Lanes 1-4 represent the ITS bands at 600bp while Lane L represents the 100bp molecular ladder.

3.4 Molecular(Genotypic) identification of lactic acid bacteria

The obtained 16S rRNA sequence from the isolates produced an exact match during the megablast search for highly similar sequences from the National Centre for Biotechnology Information (NCBI) non-redundant nucleotide (nr/nt) database. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the nine isolates, B1-B9 within Lactobacillus and *Enterobacter* sp and revealed a closely relatedness to *Lactobacillus fermentum, Lactobacillus lactis* and *Enterobacter* sp strains LAB respectively (Figure 1). The genetic sequence is shown in Table 3.

3.5 Molecular identification of yeast

The obtained ITS sequence from the isolates produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The ITS of the isolates showed a percentage similarity to other species at 99-100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of ITS of the four isolates, Y1-Y4 within the *Hypopichia*, *Pichia* and *Trichosporon* spp and revealed a closely relatedness to *Hypopichia burtonii, Pichia kudriavzevii* and *Trichosporon asahii* respectively (Fig. 1). In summary, there were 2 strains of *Hypopichia burtonii*, 1 *Pichia kudriavzevii* and 1 *Trichosporon asahii*. The genetic sequence is shown in Table 3.

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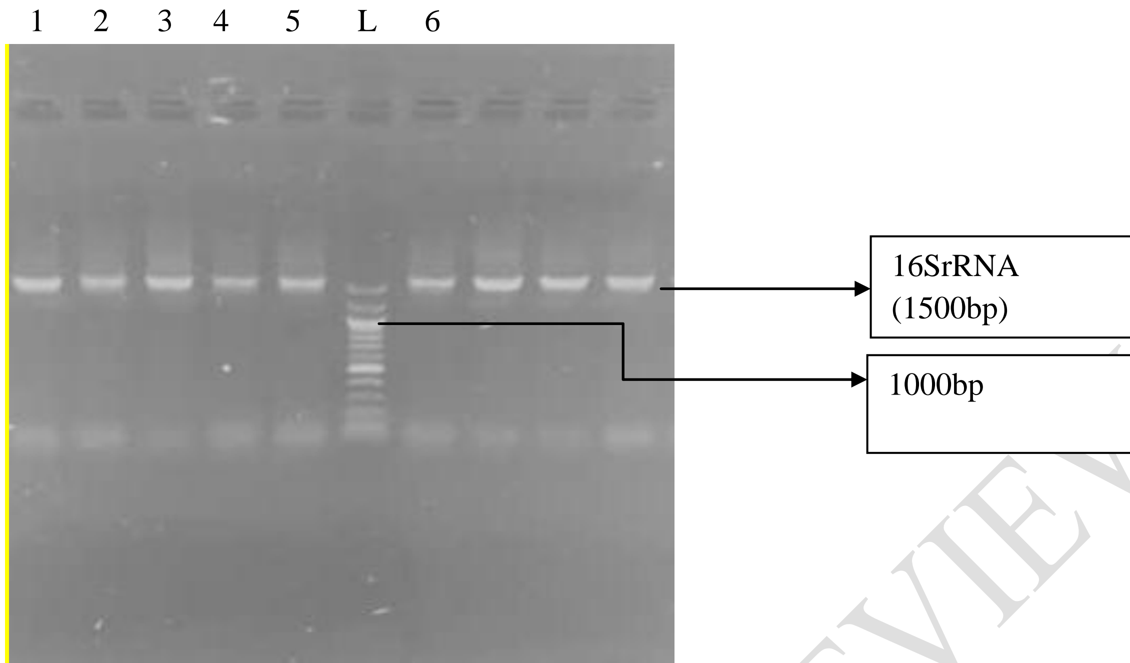

Plate 1 Agarose gel electrophoresis of the 16SrRNA of the bacterial isolates.

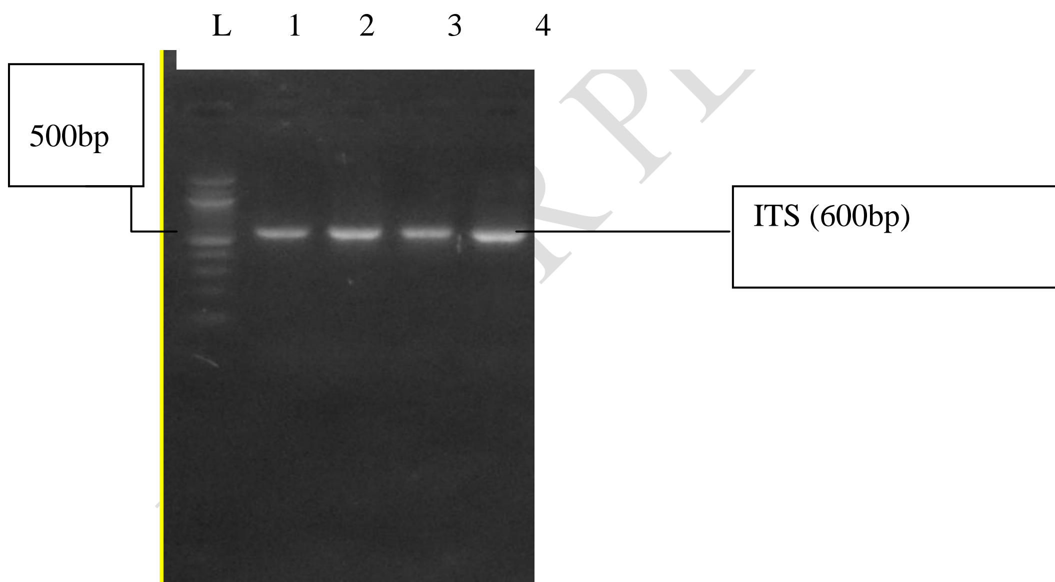

Plate 2 Agarose gel electrophoresis showing the amplified ITS of the fungal isolates.

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Table 3: Genetic Sequences of Lactic Acid Bacteria and Yeasts

	....|....| 10	....|....| 20	....|....| 30	....|....| 40	....|....| 50	....|....| 60
B1_907-R_C	GTAACGCGTG	GGGAATCTGC	CTTTGAGCGG	GGGACAACAT	TTGGAAACGA	ATGCTAATAC
B2_907-R_D	AACACGTAGG	TAACCTGCCC	AGAAGCGGGG	GACAACATTT	GGAAACAGAT	GCTAATACCG
B3_907-R_D	ACTGGATGAG	CAGCGAACGG	GTGAGTAACG	CGTGGGGAAT	CTGCCTTTGA	GCGGGGGACA
B4_907-R_D	CCAACGAGTG	GCGGACGGGT	GAGTAACACG	TAGGTAACCT	GCCCAGAAGC	GGGGGACAAC
B5_907-R_D	CCAACGAGTG	GCGGACGGGT	GAGTAACACG	TAGGTAACCT	GCCCAGAAGC	GGGGGACAAC
B6_907-R_F	AGAGCTTGCT	CTCGGGTGAC	GAGTGGCGGA	CGGGTGAGTA	ATGTCTGGGA	AACTGCCTGA
B7_907-R_D	CCAACGAGTG	GCGGACGGGT	GAGTAACACG	TAGGTAACCT	GCCCAGAAGC	GGGGGACAAC
B8_907-R_F	AGAGCTTGCT	CTCGGGTGAC	GAGTGGCGGA	CGGGTGAGTA	ATGTCTGGGA	AACTGCCTGA
B9_907-R_D	CCAACGAGTG	GCGGACGGGT	GAGTAACACG	TAGGTAACCT	GCCCAGAAGC	GGGGGACAAC
Y1_ITS-1_C	ATAAGGGCTT	ATAACTATAT	CCACTTACAC	CTGTGAACTG	TTCTACTACT	TGACGCAAGT
Y2_ITS-1_D	CACGTTTACG	TTTAACATAT	ACAACTAACC	AACTTAATTA	TGTCAACAAC	AACAACCAAA
Y3_ITS-1_B	CTACACTGCG	TGAGCGGAAC	GAAAACAACA	ACACCTAAAA	TGTGGAATAT	AGCATATAGT
Y4_ITS-1_E	ACGTTTACGT	TTAACATATA	CAACTAACCA	ACTTAATTAT	GTCAACAACA	ACAACCAAAA

	....|....| 70	....|....| 80	....|....| 90	....|....| 100	....|....| 110	....|....| 120
B1_907-R_C	CGCATAACAA	CTTTAAACAC	AAGTTTTAAG	TTTGAAAGAT	GCAATTGCAT	CACTCAAAGA
B2_907-R_D	CATAACAACG	TTGTTCGCAT	GAACAACGCT	TAAAAGATGG	CTTCTCGCTA	TCACTTCTGG
B3_907-R_D	ACATTTGGAA	ACGAATGCTA	ATACCGCATA	ACAACTTTAA	ACACAAGTTT	TAAGTTTGAA
B4_907-R_D	ATTTGGAAAC	AGATGCTAAT	ACCGCATAAC	AACGTTGTTC	GCATGAACAA	CGCTTAAAAG
B5_907-R_D	ATTTGGAAAC	AGATGCTAAT	ACCGCATAAC	AACGTTGTTC	GCATGAACAA	CGCTTAAAAG
B6_907-R_F	TGGAGGGGGA	TAACTACTGG	AAACGGTAGC	TAATACCGCA	TAACGTCGCA	AGACCAAAGA
B7_907-R_D	ATTTGGAAAC	AGATGCTAAT	ACCGCATAAC	AACGTTGTTC	GCATGAACAA	CGCTTAAAAG
B8_907-R_F	TGGAGGGGGA	TAACTACTGG	AAACGGTAGC	TAATACCGCA	TAACGTCGCA	AGACCAAAGA
B9_907-R_D	ATTTGGAAAC	AGATGCTAAT	ACCGCATAAC	AACGTTGTTC	GCATGAACAA	CGCTTAAAAG
Y1_ITS-1_C	CGAGTATTTT	TACAAACAAT	GTGTAATGAA	CGTCGTTTTA	TTATAACAAA	ATAAAACTTT
Y2_ITS-1_D	AATCAAAACT	TTCAACAACG	GATCTCTTGG	TTCTCGCATC	GATGAAGAAC	GCAGCGAAAT
Y3_ITS-1_B	CGACAAGAGA	AATCTACGAA	AAACAAACAA	AACTTTCAAC	AACGGATCTC	TTGGTTCTCG
Y4_ITS-1_E	ATCAAAACTT	TCAACAACGG	ATCTCTTGGT	TCTCGCATCG	ATGAAGAACG	CAGCGAAATG

....|....| ....|....| ....|....| ....|....| ....|....| ....|....|

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                     130       140       150       160       170       180
B1_907-R_C    TGATCCCGCG TTGTATTAGC TAGTTGGTGA GGTAAAGGCT CACCAAGGCG ATGATACATA
B2_907-R_D    ATGGACCTGC GGTGCATTAG CTTGTTGGTG GGGTAACGGC CTACCAAGGC GATGATGCAT
B3_907-R_D    AGATGCAATT GCATCACTCA AAGATGATCC CGCGTTGTAT TAGCTAGTTG GTGAGGTAAA
B4_907-R_D    ATGGCTTCTC GCTATCACTT CTGGATGGAC CTGCGGTGCA TTAGCTTGTT GGTGGGGTAA
B5_907-R_D    ATGGCTTCTC GCTATCACTT CTGGATGGAC CTGCGGTGCA TTAGCTTGTT GGTGGGGTAA
B6_907-R_F    GGGGGACCTT CGGGCCTCTT GCCATCAGAT GTGCCCAGAT GGGATTAGCT AGTAGGTGGG
B7_907-R_D    ATGGCTTCTC GCTATCACTT CTGGATGGAC CTGCGGTGCA TTAGCTTGTT GGTGGGGTAA
B8_907-R_F    GGGGGACCTT CGGGCCTCTT GCCATCAGAT GTGCCCAGAT GGGATTAGCT AGTAGGTGGG
B9_907-R_D    ATGGCTTCTC GCTATCACTT CTGGATGGAC CTGCGGTGCA TTAGCTTGTT GGTGGGGTAA
Y1_ITS-1_C    CAACAACGGA TCTCTTGGCT CTCGCATCGA TGAAGAACGC AGCGAATTGC GATAAGTAAT
Y2_ITS-1_D    GCGATAAGTA ATATGAATTG CAGATTTTCG TGAATCATCG AATCTTTGAA CGCACATTGC
Y3_ITS-1_B    CATCGATGAA GAGCGCAGCG AAATGCGATA CCTAGTGTGA ATTGCAGCCA TCGTGAATCA
Y4_ITS-1_E    CGATAAGTAA TATGAATTGC AGATTTTCGT GAATCATCGA ATCTTTGAAC GCACATTGCA

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     190       200       210       220       230       240
B1_907-R_C    GCCGACCTGA GAGGGTGATC GGCCACATTG GGACTGAGAC ACGGCCCAAA CTCCTACGGG
B2_907-R_D    AGCCGAGTTG AGAGACTGAT CGGCCACAAT GGGACTGAGA CACGGCCCAT ACTCCTACGG
B3_907-R_D    GGCTCACCAA GGCGATGATA CATAGCCGAC CTGAGAGGGT GATCGGCCAC ATTGGGACTG
B4_907-R_D    CGGCCTACCA AGGCGATGAT GCATAGCCGA GTTGAGAGAC TGATCGGCCA CAATGGGACT
B5_907-R_D    CGGCCTACCA AGGCGATGAT GCATAGCCGA GTTGAGAGAC TGATCGGCCA CAATGGGACT
B6_907-R_F    GTAACGGCTC ACCTAGGCGA CGATCCCTAG CTGGTCTGAG AGGATGACCA GCCACACTGG
B7_907-R_D    CGGCCTACCA AGGCGATGAT GCATAGCCGA GTTGAGAGAC TGATCGGCCA CAATGGGACT
B8_907-R_F    GTAACGGCTC ACCTAGGCGA CGATCCCTAG CTGGTCTGAG AGGATGACCA GCCACACTGG
B9_907-R_D    CGGCCTACCA AGGCGATGAT GCATAGCCGA GTTGAGAGAC TGATCGGCCA CAATGGGACT
Y1_ITS-1_C    GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG CAGCTTGCGC TCTCTGGTAT
Y2_ITS-1_D    ACCTTGTGGT ATTCCACAAG GTATGCCTGT TTGAGCGTCA TTTCTCCCTC AACCCCCGCG
Y3_ITS-1_B    TCGAGTTCTT GAACGCACAT TGCGCCCCTC GGCATTCCGG GGGGCATGCC TGTTTGAGCG
Y4_ITS-1_E    CCTTGTGGTA TTCCACAAGG TATGCCTGTT TGAGCGTCAT TTCTCCCTCA ACCCCCGCGG

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     250       260       270       280       290       300
B1_907-R_C    AGGCAGCAGT AGGGAATCTT CGGCAATGGA CGAAAGTCTG ACCGAGCAAC GCCGCGTGAG
B2_907-R_D    GAGGCAGCAG TAGGGAATCT TCCACAATGG GCGCAAGCCT GATGGAGCAA CACCGCGTGA
B3_907-R_D    AGACACGGCC CAAACTCCTA CGGGAGGCAG CAGTAGGGAA TCTTCGGCAA TGGACGAAAG
B4_907-R_D    GAGACACGGC CCATACTCCT ACGGGAGGCA GCAGTAGGGA ATCTTCCACA ATGGGCGCAA
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B5_907-R_D   GAGACACGGC CCATACTCCT ACGGGAGGCA GCAGTAGGGA ATCTTCCACA ATGGGCGCAA
B6_907-R_F   AACTGAGACA CGGTCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC
B7_907-R_D   GAGACACGGC CCATACTCCT ACGGGAGGCA GCAGTAGGGA ATCTTCCACA ATGGGCGCAA
B8_907-R_F   AACTGAGACA CGGTCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC
B9_907-R_D   GAGACACGGC CCATACTCCT ACGGGAGGCA GCAGTAGGGA ATCTTCCACA ATGGGCGCAA
Y1_ITS-1_C   TCCGGAGAGC ATGCCTGTTT CAGTGTCATG AAATCTCAAC CACTAGGGTT TCCTAATGGA
Y2_ITS-1_D   GGTTGGCGTT GAATGGCACG AGCTCTTAGT CAGTCCATTC GAAAAGTATT TTTCTTGTGT
Y3_ITS-1_B   TCGTTTCCAT CTTGCGCGTG CGCAGAGTTG GGGGAGCGGA GCGGACGACG TGTAAAGAGC
Y4_ITS-1_E   GTTGGCGTTG AATGGCACGA GCTCTTAGTC AGTCCATTCG AAAAGTATTT TTCTTGTGTT

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    310        320        330        340        350        360
B1_907-R_C   TGAAGAAGGT TTTCGGATCG TAAAACTCTG TTGGTAGAGA AGAACGTTGG TGAGAGTGGA
B2_907-R_D   GTGAAGAAGG GTTTCGGCTC GTAAAGCTCT GTTGTTAAAG AAGAACACGT ATGAGAGTAA
B3_907-R_D   TCTGACCGAG CAACGCCGCG TGAGTGAAGA AGGTTTTCGG ATCGTAAAAC TCTGTTGGTA
B4_907-R_D   GCCTGATGGA GCAACACCGC GTGAGTGAAG AAGGGTTTCG GCTCGTAAAG CTCTGTTGTT
B5_907-R_D   GCCTGATGGA GCAACACCGC GTGAGTGAAG AAGGGTTTCG GCTCGTAAAG CTCTGTTGTT
B6_907-R_F   GCAAGCCTGA TGCAGCCATG CCGCGTGTAT GAAGAAGGCC TTCGGGTTGT AAAGTACTTT
B7_907-R_D   GCCTGATGGA GCAACACCGC GTGAGTGAAG AAGGGTTTCG GCTCGTAAAG CTCTGTTGTT
B8_907-R_F   GCAAGCCTGA TGCAGCCATG CCGCGTGTAT GAAGAAGGCC TTCGGGTTGT AAAGTACTTT
B9_907-R_D   GCCTGATGGA GCAACACCGC GTGAGTGAAG AAGGGTTTCG GCTCGTAAAG CTCTGTTGTT
Y1_ITS-1_C   TTGGATTTGG GCGTCTGCGA TTTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA
Y2_ITS-1_D   TGTTATTTTC TAATTTAGTA GTGACAACCA CACTAAAAAC ACATTTTCCT CAAATCAGGT
Y3_ITS-1_B   GTCGGAGCTG CGACTCGCCT GAAAGGGAGC GAAGCTGGCC GAGCGAACTA GACTTTTTTT
Y4_ITS-1_E   GTTATTTTCT AATTTAGTAG TGACAACCAC ACTAAAAACA CATTTTCCTC AAATCAGGTA

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    370        380        390        400        410        420
B1_907-R_C   AAGCTCATCA AGTGACGGTA ACTACCCAGA AAGGGACGGC TAACTACGTG CCAGCAGCCG
B2_907-R_D   CTGTTCATAC GTTGACGGTA TTTAACCAGA AAGTCACGGC TAACTACGTG CCAGCAGCCG
B3_907-R_D   GAGAAGAACG TTGGTGAGAG TGGAAAGCTC ATCAAGTGAC GGTAACTACC CAGAAAGGGA
B4_907-R_D   AAAGAAGAAC ACGTATGAGA GTAACTGTTC ATACGTTGAC GGTATTTAAC CAGAAAGTCA
B5_907-R_D   AAAGAAGAAC ACGTATGAGA GTAACTGTTC ATACGTTGAC GGTATTTAAC CAGAAAGTCA
B6_907-R_F   CAGCGGGGAG GAAGGGAGTA AGGTTAATAA CCTTGTTCAT TGACGTTACC CGCAGAAGAA
B7_907-R_D   AAAGAAGAAC ACGTATGAGA GTAACTGTTC ATACGTTGAC GGTATTTAAC CAGAAAGTCA
B8_907-R_F   CAGCGGGGAG GAAGGGAGTA AGGTTAATAA CCTTGTTCAT TGACGTTACC CGCAGAAGAA
B9_907-R_D   AAAGAAGAAC ACGTATGAGA GTAACTGTTC ATACGTTGAC GGTATTTAAC CAGAAAGTCA
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Y1_ITS-1_C    TTAATGTCTG GTGTAATAAG TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT
Y2_ITS-1_D    AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAGG AA........ ..........
Y3_ITS-1_B    CAGGGACGCT TGGCGGCCGA GAGCGAGTGT TGCGAGACAA CAAAAAGCTC GACCTCAAAT
Y4_ITS-1_E    GGACTACCCG CTGAACTTAA GCATATCAAT AAGCGGAGGA A......... ..........

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     430        440        450        460        470        480
B1_907-R_C    CGGTAATACG TAGGTCCCGA GCGTTGTCCG GATTTATTGG GCGTAAAGCG AGCGCAGGTG
B2_907-R_D    CGGTAATACG TAGGTGGCAA GCGTTATCCG GATTTATTGG GCGTAAAGAG AGTGCAGGCG
B3_907-R_D    CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGTC CCGAGCGTTG TCCGGATTTA
B4_907-R_D    CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGTG GCAAGCGTTA TCCGGATTTA
B5_907-R_D    CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGTG GCAAGCGTTA TCCGGATTTA
B6_907-R_F    GCACCGGCTA ACTCCGTGCC AGCAGCCGCG GTAATACGGA GGGTGCGAGC GTTAATCGGA
B7_907-R_D    CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGTG GCAAGCGTTA TCCGGATTTA
B8_907-R_F    GCACCGGCTA ACTCCGTGCC AGCAGCCGCG GTAATACGGA GGGTGCGAGC GTTAATCGGA
B9_907-R_D    CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGTG GCAAGCGTTA TCCGGATTTA
Y1_ITS-1_C    CCGCAAGGAC AATTACTTTG ACTCTGGCCT GAAATCAGGT AGGACTACCC GCTGAACTTA
Y2_ITS-1_D    .......... .......... .......... .......... .......... ..........
Y3_ITS-1_B    CAGGTAGGAA TACCCGCTGA ACTTAAGCAT ATCAATAAGC GGAGGAA... ..........
Y4_ITS-1_E    .......... .......... .......... .......... .......... ..........

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     490        500        510        520        530        540
B1_907-R_C    GTTTATTAAG TCTGGTGTAA AAGGCAGTGG CTCAACCATT GTATGCATTG GAAACTGGTA
B2_907-R_D    GTTTTCTAAG TCTGATGTGA AAGCCTTCGG CTTAACCGGA GAAGTGCATC GGAAACTGGA
B3_907-R_D    TTGGGCGTAA AGCGAGCGCA GGTGGTTTAT TAAGTCTGGT GTAAAAGGCA GTGGCTCAAC
B4_907-R_D    TTGGGCGTAA AGAGAGTGCA GGCGGTTTTC TAAGTCTGAT GTGAAAGCCT TCGGCTTAAC
B5_907-R_D    TTGGGCGTAA AGAGAGTGCA GGCGGTTTTC TAAGTCTGAT GTGAAAGCCT TCGGCTTAAC
B6_907-R_F    ATTACTGGGC GTAAAGCGCA CGCAGGCGGT CTGTCAAGTC GGATGTGAAA TCCCCGGGCT
B7_907-R_D    TTGGGCGTAA AGAGAGTGCA GGCGGTTTTC TAAGTCTGAT GTGAAAGCCT TCGGCTTAAC
B8_907-R_F    ATTACTGGGC GTAAAGCGCA CGCAGGCGGT CTGTCAAGTC GGATGTGAAA TCCCCGGGCT
B9_907-R_D    TTGGGCGTAA AGAGAGTGCA GGCGGTTTTC TAAGTCTGAT GTGAAAGCCT TCGGCTTAAC
Y1_ITS-1_C    AGCATATCAA TAAGCGGAGG A......... .......... .......... ..........
Y2_ITS-1_D    .......... .......... .......... .......... .......... ..........
Y3_ITS-1_B    .......... .......... .......... .......... .......... ..........
Y4_ITS-1_E    .......... .......... .......... .......... .......... ..........

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                    550       560       570       580       590       600
B1_907-R_C    GACTTGAGTG CAGGAGAGGA GAGTGGAATT CCATGTGTAG CGGTGAAATG CGTAGATATA
B2_907-R_D    TAACTAGAGT ACCGAAGTGG GTAGTGGTCC TCCATCTGTA GCGGTGGAAA ACGTAGCTAT
B3_907-R_D    CATTGTATGC ATTGGAAACT GGTAGACTTG AGTGCAGGAG AGGAGAGTGG AATTCCATGT
B4_907-R_D    CGGAGAAGTG CATCGGAAAC TGGATAACTA GAGTACCGAA GTGGGTAGTG GTCCTCCATC
B5_907-R_D    CGGAGAAGTG CATCGGAAAC TGGATAACTA GAGTACCGAA GTGGGTAGTG GTCCTCCATC
B6_907-R_F    CAACCTGGGA ACTGCATCCG AAACTGGCAG GCTTGAGTCT CGTAGAGGGA GGTAGAATTC
B7_907-R_D    CGGAGAAGTG CATCGGAAAC TGGATAACTA GAGTACCGAA GTGGGTAGTG GTCCTCCATC
B8_907-R_F    CAACCTGGGA ACTGCATCCG AAACTGGCAG GCTTGAGTCT CGTAGAGGGA GGTAGAATTC
B9_907-R_D    CGGAGAAGTG CATCGGAAAC TGGATAACTA GAGTACCGAA GTGGGTAGTG GTCCTCCATC
Y1_ITS-1_C    .......... .......... .......... .......... .......... ..........
Y2_ITS-1_D    .......... .......... .......... .......... .......... ..........
Y3_ITS-1_B    .......... .......... .......... .......... .......... ..........
Y4_ITS-1_E    .......... .......... .......... .......... .......... ..........

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    610       620       630       640       650       660
B1_907-R_C    TGGAGGAACA CCGGTGGCGA AAGCGGCTCT CTGGCCTGTA ACTGACACTG AGGCTCGAAA
B2_907-R_D    ATTGAAGTAC CCCAGTGGGG AAGCCGGATC CCGGGTCTGC ATCTGCCCCT GAGCCTCGAA
B3_907-R_D    GTAGCGGTGA AATGCGTAGA TATATGGAGG AACACCGGTG GCGAAAGCGG CTCTCTGGCC
B4_907-R_D    TGTAGCGGTG GAAAACGTAG CTATATTGAA GTACCCCAGT GGGGAAGCCG GATCCCGGGT
B5_907-R_D    TGTAGCGGTG GAAAACGTAG CTATATTGAA GTACCCCAGT GGGGAAGCCG GATCCCGGGT
B6_907-R_F    CAGGTGTAGC GGTGAAATGC GTAGAGATCT GGAGGAATAC CGGTGGCGAA GGCGGCCTCC
B7_907-R_D    TGTAGCGGTG GAAAACGTAG CTATATTGAA GTACCCCAGT GGGGAAGCCG GATCCCGGGT
B8_907-R_F    CAGGTGTAGC GGTGAAATGC GTAGAGATCT GGAGGAATAC CGGTGGCGAA GGCGGCCTCC
B9_907-R_D    TGTAGCGGTG GAAAACGTAG CTATATTGAA GTACCCCAGT GGGGAAGCCG GATCCCGGGT
Y1_ITS-1_C    .......... .......... .......... .......... .......... ..........
Y2_ITS-1_D    .......... .......... .......... .......... .......... ..........
Y3_ITS-1_B    .......... .......... .......... .......... .......... ..........
Y4_ITS-1_E    .......... .......... .......... .......... .......... ..........

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    670       680       690       700       710       720
B1_907-R_C    GCGTGGGGAG CAAACAGGAT TAGATACCCT GGTAGTCCAC GCCGTAAACT ATAAGGCTAG
B2_907-R_D    AGCATGGGTA GAGACCAGGA TTAGATACCG TCGTATTCCA TGCCGTAACT TCATGAGCCA
B3_907-R_D    TGTAACTTAC ACTGAGGCTC GAAAGCGTGG GGAGCAAACA GGATTAGATA CCCTGGTAGT
B4_907-R_D    CTGCATCTGC CCCTGAGCCT CGAAAGCATG GGTAGAGACC AGGATTAGAT ACCGTCGTAT
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B5_907-R_D    CTGCATCTGC CCCTGAGCCT CGAAAGCATG GGTAGAGACC AGGATTAGAT ACCGTCGTAT
B6_907-R_F    TGGACGAAGA CTGACGCTCA GGTGCGAAAG CGTGGGGAGC AAACAGGATT AGATACCCTG
B7_907-R_D    CTGCATCTGC CCCTGAGCCT CGAAAGCATG GGTAGAGACC AGGATTAGAT ACCGTCGTAT
B8_907-R_F    TGGACGAAGA CTGACGCTCA GGTGCGAAAG CGTGGGGAGC AAACAGGATT AGATACCCTG
B9_907-R_D    CTGCATCTGC CCCTGAGCCT CGAAAGCATG GGTAGAGACC AGGATTAGAT ACCGTCGTAT
Y1_ITS-1_C    .......... .......... .......... .......... .......... ..........
Y2_ITS-1_D    .......... .......... .......... .......... .......... ..........
Y3_ITS-1_B    .......... .......... .......... .......... .......... ..........
Y4_ITS-1_E    .......... .......... .......... .......... .......... ..........

              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....
                     730        740        750        760        770
B1_907-R_C    ATGTAGGGAG CTATAAGTTC TCTGTATCGC AGCTAACGCA ATAAGCATCT CCCCTTTTT
B2_907-R_D    GCATGAAGCC GGGCTCGGCC CCTCCGTGCC GCAGCAAATG CAGCAACATT CTCCCACCT
B3_907-R_D    CCACGCCGTA AACTATAAGG CTAGATGTAG GGAGCTATAA GTTCTCTGTA TCGCAGCTA
B4_907-R_D    TCCATGCGGT AACTTCATGA GCCAGCATGA AGCCGGGCTC GGCCCCTCCG TGCCGCAGC
B5_907-R_D    TCCATGCGGT AACTTCATGA GCCAGCATGA AGCCGGGCTC GGCCCCTCCG TGCCGCAGC
B6_907-R_F    GTAGTCCACG CCGTAAACGA TGTCTATTTG GAGGTTGTGC CCTGAGGCGT GGCTTCCGG
B7_907-R_D    TCCATGCGGT AACTTCATGA GCCAGCATGA AGCCGGGCTC GGCCCCTCCG TGCCGCAGC
B8_907-R_F    GTAGTCCGCG CCGTAAACGA TGTCTATTTG GAGGTTGTGC CCTGAGGCGT GGCTTCCGG
B9_907-R_D    TCCATGCGGT AACTTCATGA GCCAGCATGA AGCCGGGCTC GGCCCCTCCG TGCCGCAGC
Y1_ITS-1_C    .......... .......... .......... .......... .......... .........
Y2_ITS-1_D    .......... .......... .......... .......... .......... .........
Y3_ITS-1_B    .......... .......... .......... .......... .......... .........
Y4_ITS-1_E    .......... .......... .......... .......... .......... .........
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UNDER REVIEW

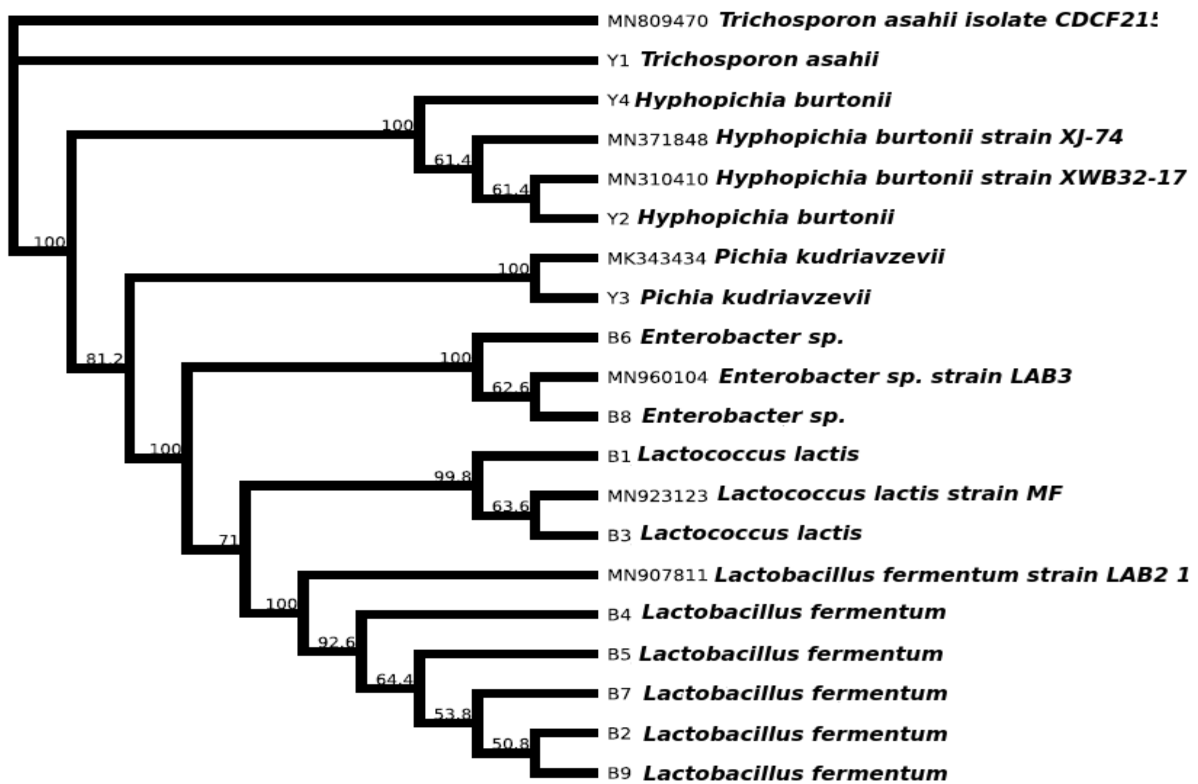

Figure 1: Phylogenetic tree showing the evolutionary distances between the bacterial and yeast isolates

UNDER PEER

4. Discussion

Lactic acid bacteria (LAB) and yeasts are involved in the spontaneous fermentations of cereals in synergy, where the yeasts help to produce high levels of ethanol and confer desirable flavour as well as providing some proteins for LAB growth, while the LAB principally produce the desirable acids for preservation and acidic environment for yeast growth. [21,22].

In this study, the LAB isolated from the 'ogi' (fermented maize and sorghum blend) were characterized using phenotypic and genotypic methods. It was established that *Lactobacillus fermentum* is the dominant flora as five strains were identified. They were also dominant in the production of lactic acid (1.54%) and tolerated a low pH of 4.4. Houngbedji et al. [22] likewise identified *Lactobacillus fermentum* as the dominant flora in the fermented cereal

dough, 'mawe' consumed in Benin. In the study conducted by Olatunde et al. [23] 88 LAB were isolated from effluents generated during 'ogi' production of which *Lactobacillus* spp. predominated in the lactic acid production. Agati et al. [5] isolated 12 LAB with amylolytic abilities from fermented cereal dough, which also included *Lactobacillus fermentum* as reported in the present study. Thus, in agreement with these and other works [3-5,24], this study also confirmed that some strains of *Lactobacillus fermentum* are amongst key players in the fermentation of ogi.

The other lactic acid bacteria isolated were two strains of *Lactococcus lactis* and *Enterobacter sp.* The isolates were able to grow at 45°C, a low pH of 4.4 and produced lactic acids ranging from 0.27-1.22%. They also contributed to the acidification of the medium.

The yeast isolates were strains of *Hypopichia burtonii*, *Pichia kudriavzevii* and *Trichosporon asahii*. The yeast isolates were tolerant to ethanol except *Pichia kudriavzevii.* The yeast, *Pichia kudriavzevii* has been reported for its qualities as a probiotic, flavour enhancer, preservative as well as the ability to withstand a low pH and remaining metabolically active at 45°C [25]. Houngbedji et al. [22] identified *Pichia kudriavzevii* as the predominant yeast involved in the spontaneous fermentation of cereal-based dough. *Trichosporon asahii* is among dominant yeasts in the production of *Fura*, a spontaneously fermented pearl millet product consumed in West Africa [26] and *Nono*, a traditional fermented milk [27]. *Hypopichia burtonii* is commonly associated with the fermentation of rice and coffee [28,29].

The LAB are dominant players in fermenting food matrix like cereal-porridge, supported by the yeast as confirmed in this study. Since the isolated LAB and yeasts were characterized with phenotypic and genotypic methods, the correct identities of the isolates were ascertained, and informed that the phenotypic methods should be supported with the genotypic ones as suggested in other studies [24,30].

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5. Conclusion

The strains of LAB isolated in this study have been reported as strains of probiotic bacteria. The yeasts have also been reported as good candidates as starter cultures in cereal fermentation. In combination, the LAB and the yeast isolates can be used as starter cultures to produce 'ogi' of desirable nutritional, microbiological and organoleptic qualities under and safe controlled fermentation processes.

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COMPETING INTERESTS DISCLAIMER:

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