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Molecular identification and technological properties of yeasts isolated from spontaneously fermented cassava waste pulp

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Abstract

The aim of this work was to report on molecular identification and technological properties of the yeast flora isolated from spontaneously fermented cassava waste pulp. This was done with a view of obtaining yeast strains that could be used as a starter culture for the fermentation of cassava waste pulp. Molecular identification was based on the nucleotide sequence of the ITS region of the genomic DNA of the yeast isolates while the technological properties evaluated include linamarase (UmL^{-1}), gelatinase, and haemolytic activity, growth at pH 2.5, and tolerance to 2 % bile salt. All the representative five isolated yeasts were identified as *Geotrichum silvicola* KLP04, KLP06, KLP07, KLP08 and *Geotrichum candidum* KLP05. The isolates exhibited linamarase activity ranging between 3.3 and 4.2 with strain KLP04 having the highest value and strain KLP05 the least. None of the isolates demonstrated gelatinase and haemolytic activity except strain *Geotrichum silvicola* KLP08 which was partially haemolytic. All the examined yeasts exhibited good growth at pH 2.5, with strain KLP08 having the highest viable counts of 4.1 $\log_{10}cfumL^{-1}$ and strain KLP04 the least value of 3.5 $\log_{10}cfumL^{-1}$ after 72 h of growth. All the identified yeasts showed strain-specific tolerance to 2 % bile salt with strain KLP04 having the highest viable count of 4.3 $\log_{10}cfumL^{-1}$ and strain KLP08 the least value of 2.2 $\log_{10}cfumL^{-1}$ at the end of 72 h of incubation. Based on all the examined technological properties, *Geotrichum silvicola* KLP04 strain had the highest potential to be considered for starter culture for the fermentation of cassava waste pulp.

Introduction

The maize grain is a major feed grain and a standard component of livestock diets where it serves as a source of energy (Heuzé *et al.* 2017). For instance, its proportion in monogastric tropical diets could range between 50 and 70 % (PAN 1995). However, the high consumption of maize both by the human population and the livestock industries coupled with its low level of production have continuously generated a demand-supply gap with a concomitant price increase. The attendant effect of this scenario on the increased cost of animal feeds has been an age-long one for which cheaper alternative feedstuffs have been developed to replace the expensive conventional ones (Salami and Odunsi 2003). Due to their cheapness, agricultural wastes and by-products of food processing have become the first line of choice as unconventional feed materials in livestock

industries. Examples of these materials include sorghum spent grains, wheat offals as well as cassava by-products. According to Lukuyu *et al.* (2014), cassava by-products that have found application in feeding livestock include cassava leaf, cassava leaf meal, cassava leaf protein concentrate, cassava peels, cassava stumps, cassava sievate and cassava pomace/pulp/bagasse/starch residue.

Cassava meal provides dietary energy to over 500 million people in the world (ARC 2014). FAO (2001) reported that the global production of cassava in the year 2000 was 172 million tons with Africa accounting for 45 %, Asia 28 %, and Latin America and the Caribbean 19 %. Nigeria, Brazil, Thailand, Congo (DRC), and Indonesia are the five top producing countries. Current statistics show that Nigeria still accounts for 20 % of cassava global production (FAO 2020). In contrast to Latin America and Southeast Asia, where the majority of cassava is exported for industrial purposes or animal feed; about 70 to 80 % of cassava produced in Nigeria is utilized for human consumption (Dada *et al.* 2010) and only a reported 5 % of cassava was used as livestock feed (Apata and Babalola 2012). However, in recent times, the industrial potential of cassava to produce starches for textiles, pharmaceutical, food, alcohol, acetone, and dextrin industries are largely being exploited. This current trend is adding another stream of cassava waste to those that have been known to be generated from the processing of cassava tuber for human food. It has been estimated that the supply-demand gap for cassava starch and high-quality cassava flour in Nigeria are 290,000 and 485,000 metric tons (MT) per year, respectively (PWC 2020). This corresponds to the respective estimated generation of 870,000 and 485,000 MT of cassava pulp as waste annually.

The high cyanogenic glycosides contents as well as low protein content of cassava wastes constitute a restraint to their full exploitation as livestock feed. Different processes found to be effective in reducing cyanogenic glycosides include sun-drying, ensiling and soaking plus drying (Tewe 1992; Salami and Odunsi 2003). In addition, fermentation of cassava products and by-product with starter microorganisms such as *Saccharomyces cerevisiae* and *Lactobacillus* spp. (Oboh 2006; Ubalua 2007), *Trichoderma viride* (Ezekiel *et al.* 2010), *Aspergillus niger* and *Saccharomyces cerevisiae* (Iyayi and Losel 2001), *Rhizopus oligosporus* and *Aspergillus niger* (Kolapo *et al.* 2021), and *Rhizopus oryzae* (Vlavonou 1988) resulted in a product with higher protein content, lower cyanogenic glycosides and phytate content. In addition, the inclusion of microorganisms with probiotic potentials to the animal diet is known to promote growth and enhance the performance of livestock (Nagpal *et al.* 2015; Arowolo and He 2018). In this regard, some yeasts, such as *Saccharomyces cerevisiae* (Alizadeh *et al.* 2016), selenium yeast and *Phaffia rhodozyma* yeast (Shurson 2018) have shown remarkable usefulness.

Utilization of cassava and its wastes for livestock feeding has long been realized as various reports on their use for feeding poultry (Ravindran 1991; Salami and Odunsi 2003; Adeyemo *et al.* 2014; Omede *et al.* 2017), pigs (Iyayi and Tewe 1988; Unigwe *et al.* 2014) aquaculture (Solomon *et al.* 1999; Okoli 2020) and ruminants (Smith 1988; Lukuyu *et al.* 2014; Oloruntola *et al.* 2019) have been documented. In addition, with the increasing demand for cassava starch and high-quality cassava flour in Nigeria, many medium and large-scale industries are being attracted to the cassava starch and flour processing sector. In Nigeria, the cassava flour industries have been estimated to generate 870,000 and 485,000 MT of cassava waste pulp annually. Like previous cassava wastes generated from processing of cassava for human food, appropriate technologies might be deployed to ensure that this new stream of waste (cassava waste pulp) is incorporated into livestock feeding programs. In order to achieve this, the understanding of the yeasts profile and their technological properties of spontaneously fermented cassava waste pulp is necessary. To the best of our knowledge, investigation which focused on molecular identification of the yeast profile of spontaneously fermented cassava waste pulp has not been reported. Thus, this study aimed to report on molecular identification and technological properties of the yeast flora isolated from spontaneously fermented cassava waste pulp. This was done with a view of obtaining yeast strains that

could be used as starter culture for the fermentation of cassava waste pulp.

Experimental

Preparation and spontaneous fermentation of cassava waste pulp

Freshly harvested cassava tubers of the TMS 92/0067 variety were obtained from the Root and Tuber Expansion Programme of the International Institute of Tropical Agriculture, located in Ogere, Ogun State, Nigeria. The cassava tubers were harvested after 12 months of planting.
The tubers were peeled by removing both the bark (outermost thin brown layer) and the cortex of cassava tuber. After peeling, 1 kg of cassava tubers was washed and grated using a mechanical grater to obtain cassava pulp. The pulp was mixed with 0.5 L of clean tap water and stirred vigorously. The resulting suspension was screened using a double layer of cheese cloth to extract the cassava starch. The residual mass was rinsed with excess water for about three times to extract as much starch as possible. The residue left thereafter was cassava waste pulp (CWP). The CWP was divided into three portions. Each portion was packed into a salt bag and the mouth tied up and left for 7 days for spontaneous fermentation to take place at the ambient temperature (30 ºC).

Isolation of microorganisms

Ten grams (10 g) of fermented CWP from each of the triplicate setups were homogenized in 90 mL of sterile buffered peptone water. The three homogenates were ten-fold serially diluted using the same diluent. Aliquots of serially diluted samples were pour-plated on Yeast and Mould Agar supplemented with 100 $mg.L^{-1}$ chloramphenicol (Oxoid, Basingstoke Hampshire, UK). Distinct colonies were streaked on the medium of isolation twice to obtain pure cultures (Schwan *et al.* 2007). The ability of all the yeast isolates to produce linamarase enzyme was evaluated (as described in the subsequent section). Pure cultures of yeast isolates with significant linamarase activities were selected and maintained on agar slants at 4 ºC for further molecular characterization studies.

Preliminary characterization of yeast isolates

Yeast isolates were assessed macroscopically for colour and appearance. A microscopic examination was done to assess the presence/absence of hyphae and arthrospores.

Molecular identification of isolates

Genomic DNA extraction from overnight cultures of the isolates grown in Luria-Bertani broth (Acumedia, Michigan, USA) was performed by using the ZR Fungal/Bacterial DNA MiniPrep™ Kit (Zymo Research, California, USA) as described by Adedeji *et al.* (2017). Fresh cultures were centrifuged at 10,000 × g for 1 min. The microbial cells were lysed by bead beating in a lysis buffer, and the lysate was subsequently centrifuged. The supernatant was passed through a column matrix to allow for DNA binding. The bound DNA was purified and eluted from the column matrix. Agarose gel electrophoresis technique was used to verify the integrity of the eluted DNA, while quantification was carried out using Qubit 2.0 fluorometer (Thermo Fisher Scientific, Massachusetts, USA).
PCR amplification of the internal transcribed spacer (ITS) region of genomic DNA of the yeast isolates was done using primers ITS1: 5′ TCCGTAGGTGAACCTGCGG 3′ and ITS4: 5′ TCCTCCGCTTATTGATATGC 3′ (White *et al.* 1990). PCR was performed in a total volume of 50 µL containing 30 – 50 ng DNA, 100 mM of each primer, 0.05 $U.\mu L^{-1}$ *Taq* DNA polymerase, 4 mM $MgCl_2$, and 0.4 mM of each dNTP. The amplification reaction was performed with a C1000 Touch thermal cycler (Bio-Rad Laboratories, California, USA). The thermal cycling condition used was an initial denaturation at 96 °C for 5 min, followed by 30 cycles of denaturation at 96 °C for 45 s, annealing at 56 °C for 30 s and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 5 min and a holding period at 4 °C for infinite time. The PCR amplicons were analysed by electrophoresis in 1 % (w/v) agarose gel with ethidium bromide, 1 kb DNA ladders were loaded

in 5 µL volumes, while 7 µL of the sample was loaded with 2 µL of loading dye. The gel was allowed to run for 2 h at 60 V. Gel results were visualized with a ChemiDoc™ MP System (Bio-Rad Laboratories, California, USA) to confirm the expected size of the product. The remaining PCR products were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany).

The sequencing of the purified PCR products was done with PRISM™ Ready Reaction Dye Terminator Cycle Sequencing Kit using the dideoxy chain termination method and electrophoresed with the model ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. ChromasLite version 2.33 software was used for the analysis of electropherograms, (sense and antisense) resulting from sequencing reaction for good quality sequence assurance. The resulting electropherograms were edited using BioEdit Sequence Alignment Editor. After this, the resulting consensus sequences obtained were Blast in the NCBI (www.ncbi.nlm.nih.gov) database with the Basic Alignment Search Tool (BLASTn) for homology in order to identify the probable organism in question (Dabassa *et al.* 2019). These sequences were deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers MW232914, MW233017, MW233034, MW233050, and OK070746.

Phylogenetic analysis

The phylogenetic analysis was based on the sequence of each species in order to establish relationships among them. The evolutionary history was inferred by using the Maximum Likelihood method and the Hasegawa-Kishino-Yano model (Hasegawa *et al.* 1985). The tree with the highest log likelihood (-2931.96) was shown. The percentage of trees in which the associated taxa clustered together was shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 6 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. There was a total of 485 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018).

Evaluation of technological properties of isolated yeasts

Linamarase activity

The method described by Oyewole (2001) and O'Brien *et al.* (1991) was used to evaluate the ability of the yeast isolates to produce linamarase (EC 3.2.1.21). Linamarase activity of the isolates was quantitatively determined as β-glucosidase glycohydrolase. The estimation was carried out by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucoside using a spectrophotometer. A loop full of 18h culture was added to 6 mL of 10g.L^{-1} of *p*-nitrophenyl-β-D-glucoside in 50 g.L^{-1} sodium citrate (pH 6.0) and incubated at 37 ºC for 15 min. Three milliliters of 0.1 M sodium carbonate (Na_2CO_3) were subsequently added to terminate the reaction. The absorbance was then measured at 420 nm using the Jenway model 6305 UV/VIS spectrophotometer. A standard curve enabled the conversion of the absorbance obtained to the quantity of *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucoside. One unit of activity was defined as the amount releasing 1 µmol of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucoside in 1 min under the assay condition.

Haemolytic and gelatinase activity

The yeast isolates were cultured in Malt Extract Broth at 37 °C for 12 – 18 h and then transferred onto blood agar (Difco, Michigan, USA) plates supplemented with 5 % defibrinated whole sheep blood as described by Yoon *et al.* (2008). After 24 – 48 h, the plates were examined for haemolytic reaction. A partial lysis of red blood cells and greening zone was recorded as α-haemolysis while a clear zone around yeast growth was taken as

β-haemolysis and no lysis was recorded as γ-haemolysis.
Nutrient gelatin medium, consisting of gelatin, peptone, and beef extract was used for the determination of gelatinase activity. 3 mL of gelatin medium that had been previously adjusted to pH 6.8 using 0.1 M NaOH was dispensed into test tubes which were then autoclaved at 121 ºC for 15 min. The tubes were allowed to cool in an upright position. For 24 h, yeast cultures were inoculated into the tubes and incubated at 35 ºC for 48 h. A positive result was indicated by partial or complete liquefaction of the inoculated tube at 4 ºC. In a tube with a negative result, the content of the tube remained completely solidified at the end of refrigeration.

Growth at low pH

The ability of the yeast isolates to grow at low pH was evaluated using the method described by Conway *et al.* (1987). Fresh culture of yeast strains was inoculated into Malt Extract Broth (1 % v/v) with pH adjusted to 2.5 using 3N HCl. The inoculated broth was then incubated at 37 ºC for 72 h. Viable cell counts of samples that were aseptically taken at 0, 24, 48, and 72 h were determined. Each determination was carried out in triplicates.

Tolerance to bile salts

The ability of the strains to grow in the presence of bile was determined according to the method of Conway *et al.* (1987). The procedure was carried out by inoculating 1% (v/v) fresh culture of selected yeast strains into Malt Extract Broth supplemented with 2% bile salts. This was followed by incubation at 37ºC for 72 h. Viable cell counts were determined at 0, 24, 48, and 72 h. Each determination was carried out in triplicates.

Statistical Analysis

Data obtained was expressed as means ± standard deviation. Analysis of variance was carried out on the data obtained to determine the significance of differences. A two-tailed P-value of less than 0.05 was considered as statistically significant. Values that were significantly different were separated using the Duncan Multiple Range test using SPSS for windows Verson 17.0 statistical package.

Results and Discussion

A total of nineteen yeast isolates were obtained from the three samples of the investigated naturally fermented cassava waste pulp. Out of these, five isolates that demonstrated significant linamarase activity were further studied. The macroscopic appearance of the yeast isolates was creamy white with a glossy surface. Microscopic examination of the isolates revealed the presence of true hyphae and arthrospores.
The blast of the sequences of yeasts isolated from naturally fermented Cassava Waste Pulp is shown in Table 1. The four isolates from this study were identified as *Geotrichum silvicola* while the fifth isolate was identified as *Geotrichum candidum.* Fig.1 shows the maximum likelihood phylogenetic tree. It describes the evolutionary relationship between and among strains of organisms based on their character. The marked strains denoted with, KLP04, KLP05, KLP06, KLP07 and KLP08, are those from the present study while the others are the related/similar strains retrieved from the GenBank. As shown in the tree, the strains from this study are not closely related to the strains already deposited in GenBank as the degree of relatedness (homology) they shared with the reference strains is less than 70 %, even though they share relatively high similarity (Stackebrandt and Goebel 1994).
A previous report on phenotypic characterization of yeasts presented in some Nigerian traditional fermented foods such as burukutu, ogi, and pito confirmed the presence of *Candida glabrata*, *Debaryomyces hansenii*, *Candida krusei*, *Candida colliculosa*, *Pichia anomala*, *Pichia farinosa*, and *Pichia membranefaciens* (Alakeji *et al.* 2015). In a related development, Omemu *et al.* (2007) have isolated *Saccharomyces cerevisiae, Candida krusei, Candida tropicalis, Geotrichum candidum, Geotrichum fermentans,* and *Rhodotorula graminis* during the fermentation of maize for *ogi* production. Taxonomic characterization based on morphological, physiological, and biochemical data indicated the presence of *Rhodotorula glutinis*

(Fresenius) F. C. Harrison var. *glutinis* – a linamarin degrading yeast in cassava wastewater Previous characterization of yeast ecology of fermented cassava products has documented the presence of different species of the following genera: *Saccharomyces, Candida, Hansenula, Penicillium, Geotrichum, Rhodotorula, Pichia*, and *Zygosaccharomyces* (Amoa-Awua *et al.* 1997; Oyewole 2001; Coulin *et al.* 2006; Schwan *et al.* 2007). In another development, *Galactomyces* spp was reported to be one of the yeasts involved in the fermentation of sour cassava starch (Lacerda *et al.* 2005). However, *Geotrichum silvicola* sp. nov. has been described as a novel asexual arthroconidial yeast species related to the genus *Galactomyces* (Pimenta *et al.* 2005). Recently, *Geotrichum silvicola* has been isolated from spontaneously fermenting *motoho*, a southern African non-alcoholic sorghum beverage (Moodley *et al.* 2019). The present study is the first to report the presence of *Geotrichum silvicola* in cassava fermented products.

treatment lagoons (Vasconcellos *et al.* 2009).

Table 1. Molecular identification of yeasts isolated from spontaneously fermented cassava waste pulp based on the ITS region of the genomic DNA.

Isolate Code	Identity	Organisms in GenBank with significant alignment with isolate	Max. score	Total score	Query cover	Identity [%]	Accession number
KLP04	*Geotrichum silvicola*	*Geotrichum silvicola* CBS 9194	500	500	95	96.43	MW232914
KLP05	*Geotrichum candidum*	*Geotrichum candidum AUMC10284*	472	472	98	93.48	OK070746
KLP06	*Geotrichum silvicola*	*Geotrichum silvicola* CBS 9194	478	478	95	93.75	MW233017
KLP07	*Geotrichum silvicola*	*Geotrichum silvicola* CBS 9194	461	461	95	92.90	MW233034
KLP08	*Geotrichum silvicola*	*Geotrichum silvicola* CBS 9194	572	572	90	97.36	MW233050

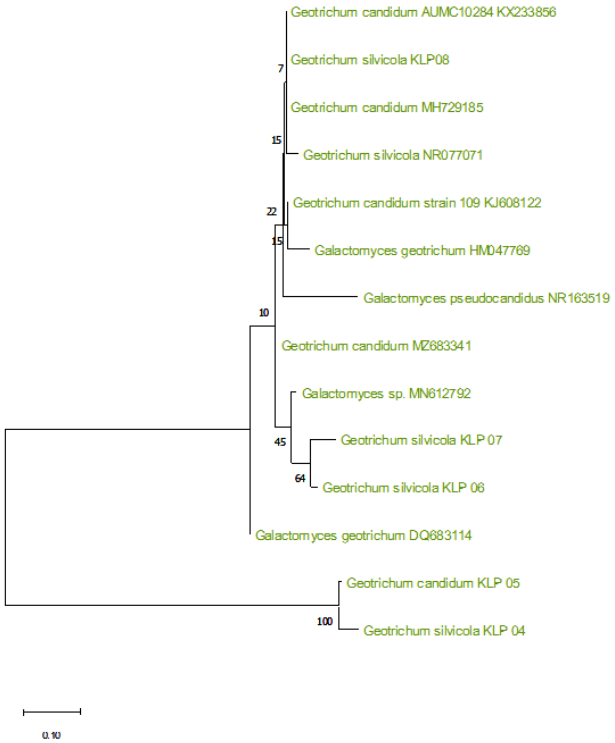

Fig. 1. The maximum likelihood phylogenetic tree of the sequenced isolates and other reference strains retrieved from GenBank (*strains ending with KLP 04, KLP05, KLP06, KLP07, and KLP08 are isolates from the present study).

The linamarase activity (UmL^{-1}) of the yeast isolated from fermented cassava waste pulp is shown in Table 2. The isolates exhibited activity ranging between 3.3 and 4.2 with strain KLP04 having the highest value and strain KLP05 the least. There is no significance difference among KLP06, KLP07, and KLP08. The value obtained in this report is within the range (0.5 – 5.8) reported by Nwokoro and Anya (2011) for different fractions of linamarase enzyme of *Lactobacillus delbrueckii* NRRL B-763. Various workers have reported that yeasts and lactic acid bacteria are the major microorganisms responsible for the fermentation of cassava (Essers *et al.* 1995; Amoa-Awua *et al.* 1996; Kobawila *et al.* 2005). The exhibited high linamarase activity of the isolated yeasts in the present study is indicating their capabilities to degrade the cyanogenic glycosides in cassava waste pulp; hence they could be used as starter cultures to produce fermented cassava waste pulp.

Table 2. Linamarase ($U.mL_{-1}$), gelatinase and haemolytic activity of yeasts isolated from fermented cassava waste pulp.

Isolate code	Linamarase activity	Gelatinase activity	Haemolytic activity
KLP04	4.2 ± 0.2^{a}	Negative	γ-haemolysis
KLP05	3.3 ± 0.1^{c}	Negative	γ-haemolysis
KLP06	3.7 ± 0.1^{b}	Negative	γ-haemolysis
KLP07	3.6 ± 0.0^{b}	Negative	γ-haemolysis
KLP08	3.6 ± 0.1^{b}	Negative	α -haemolysis

Values are means ± standard deviation (n = 3). Within each column, values with different superscripts are significantly different.

The gelatinase and haemolytic activities of the isolated yeasts are shown in Table 2. None of the isolates demonstrated the two activities except strain KLP08, which was partially haemolytic. The results obtained in this study, except for strain KLP08, are similar to the findings of Franz *et al.* (2001), Mannu *et al.* (2003) and Banwo *et al.* (2012) on enterococci of food origin. Gelatinase genes (Gel gene) may be silent, and phenotypes may be negative, even in the presence of Gel gene (Franz *et al.* 2001; Yoon *et al.* 2008). Most yeast present in food satisfies the important criteria of safety due to long history of safe human consumption in traditional fermented food products. Hence, yeasts have the Generally Regarded as Safe (GRAS) status (FDA 2001). All yeast isolates (except strain KLP08) in the present study could be tentatively assigned GRAS status as they exhibited safety potentials. The result of the present study underscores the absolute necessity to establish the safety status of every strain that will be used for food fermentation as intra-species variation was observed for haemolytic activity of the isolated yeasts.

The quantitative determination of the effect of different conditions such as pH 2.5 and 2 % bile salt on the growth pattern of the isolated yeasts revealed strain-specific responses. All the examined yeasts exhibited good growth at pH 2.5 after the intervals of 24, 48, and 72 h (Fig. 2). At the end of 72 h of growth, strain KLP08 showed the highest viable counts of 4.1 $\log_{10}cfu.mL^{-1}$ and strain KLP04 the least value of 3.5 $\log_{10}cfu.mL^{-1}$. These results are comparable to the findings of Alakeji *et al.* (2015), who reported highest (6.78 $\log_{10}cfu.mL^{-1}$) and least (2.18 $\log_{10}cfu.mL^{-1}$) viable counts for *Candida colliculosa* PII and *Pichia membranefaciens* BA2 respectively after grew at pH 2.5 for 72 h. In a related development, Psomas *et al.* (2001) and Kourelis *et al.* (2010) reported that strains of *Saccharomyces cerevisiae*, *Candida albicans* and *Debaryomyces hansenii* have survived at pH 3.0. From the results obtained for the influence of 2 % bile salt on the growth pattern of the examined yeast (Fig. 3), all the yeasts showed strain-specific tolerance to 2 % bile salt. In this regard, strain KLP04 had the highest viable count 4.3 $\log_{10}cfu.mL^{-1}$ and strain KLP08 the least value 2.2 $\log_{10}cfu.mL^{-1}$ at the end of 72 h of incubation. *Debaryomyces hansenii* OA3 and *Candida glabrata* SPY3 were reported to have the highest (6.25 $\log_{10}cfu.mL^{-1}$) and least (1.88 $\log_{10}cfu.mL^{-1}$) viable counts respectively after grew under 2 % bile salts for 72 h (Alakeji *et al.* 2015). Similarly, in-vitro studies have shown that *Wickerhamomyces anomalus* survived excellently in >0.6 % bile salts (Garcia-Hernadenz *et al.* 2012; Bonatsou *et al.* 2015).

Klaenhammer and Kullen (1999) had stated that important criteria for the selection of probiotic strains are the maintenance of their cell integrity and retaining of their beneficial metabolic functions during gastrointestinal passage.

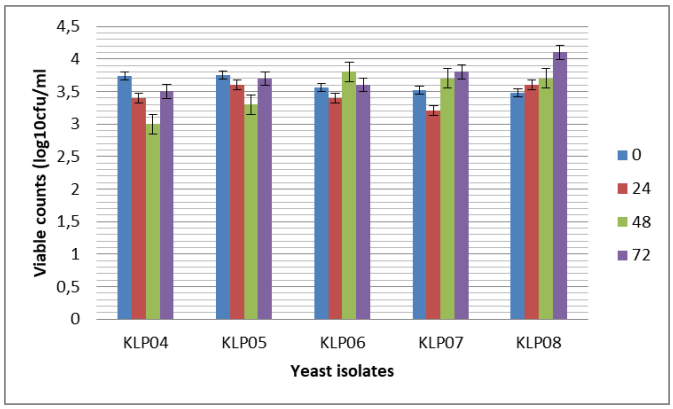

Fig. 2. Effect of pH 2.5 on the growth of selected yeast strains.

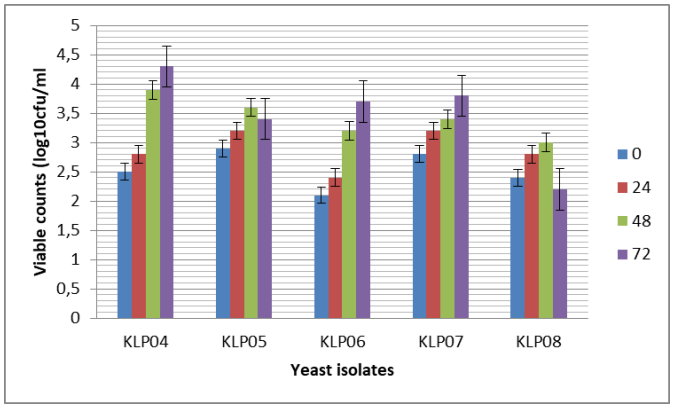

Fig. 3. Effect of 2 % bile salts on the growth of selected yeast strains.

In this connection, there is the need for the strains to survive the natural barriers in the intestine, including body temperature, low pH and elevated bile concentration, as Czerucka *et al.* (2007) had stated that the stomach pH at fed state could be as low as 2.5 for 3 h. All the isolated yeasts examined in the present study had impressive growth at the body temperature of 37 ºC, low pH of 2.5 and under 2 % bile salt condition. This may confirm that they are eminently qualified as probable probiotic that could be used for the fermentation of cassava waste pulp for the production of animal feed. However, further consideration of their safety potentials may disqualify strain KLP08 on the ground of being partially haemolytic.

Conclusion

Based on all the technological properties examined in this study, *Geotrichum silvicola* KLP04 strain had the highest potential to be considered for starter culture for the fermentation of cassava waste pulp as it demonstrated the highest linamarase activity and tolerance to 2 % bile salt in addition to having good growth at pH 2.5 and demonstration of GRAS status.

Conflict of Interest

The authors declare that they have no conflict of interest.

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