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Research Article

Characterization of Wild Yeasts Isolated From Banana Fruit and Honey for Wine Production

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ABSTRACT

Occupational oenologists have long used specific genera of yeast for alcoholic fermentation of wine; thus, the characterization of yeasts is of great importance to oenologists. In this study, commercial wine yeast (CY) with the GV1 remark and wild yeasts isolated from honey, banana fruit were characterized using standard microbiological methods. The local BLAST of the Analytical Profile Index (API) kit confirmed that the isolates (B^7, B^{10}, H^7, H^4 and CY) had 99.90% relatedness to *Saccharomyces cerevisiae, Candida tropicalis, Saccharomyces cerevisiae, Candida hellenica* and *Candida kefyr*, respectively; while molecular characterization revealed that isolates accession number MK752669, EF190223, EF198007, KF268353 and EF192229 had 93.10%, 95.00%, 94.80%, 77.60% and 92.10% relatedness to *Candida tropicalis* strain Pe 1, *Candida tropicalis* strain WC65-1, *Candida tropicalis* strain WC57, *Clavispora lusitaniae* strain WM03, and *Candida tropicalis* strain zhuan4, respectively. The polymorphism of the length of the restriction fragments revealed the disparity between the yeasts by indicating their molecular weight, which ranges from 400 – 600 basepairs (bp) on the molecular ladder. Wild yeasts possess Alcohol O-acetyltransferase (ATF) 1 gene, Pyruvate decarboxylase 1 (PDC 1) gene, Branched-chain amino acid transaminase (BAT) 1 gene, Thiamine pyrophosphate-dependent 2-oxo-acid decarboxylase (ARO) 10 gene, and Alcohol dehydrogenase (ADH) 1 and 2 genes in their chromosomal DNA, except isolate B10 that lack the PDC1 and ARO 10 genes. This study revealed that some locally sourced yeast isolates possess essential functional genes needed for quality wine production such as the imported commercial wine yeast.

Keywords: Honey, Banana, wild yeasts, Commercial wine yeasts, Must, Alcohol dehydrogenase (ADH) 1 and 2 genes.

Introduction

For thousands of years, the techniques involved in the production of wine have been in use, but the microbiological features underlying the processes such as the identification of yeast as winemakers and the fundamental aspects of alcoholic fermentation are relatively being understood recently; and the quality of wine has improved greatly since Pasteur's findings: which is attributed to increased knowledge acquired from the physiology and genetics of yeast as the choice of wine yeast to influence the quality and flavours of wine (Grossmann *et al*., 2011). Masneuf-Pomarede *et al*. (2016) confirmed that in wine production, alcoholic fermentation is accomplished by *Saccharomyces cerevisiae* as conventional wine yeast that possesses oenological characteristics such as complete alcoholic fermentation, the low release of off-flavour compounds, and positive impact on wine aromas. However, there is growing research focusing on the isolation and characterization of wine yeasts which could offer varieties of wine with specific requirements to meet consumer's demand for reduced alcohol content, which has led to the exploration of new species of yeast (non-conventional yeasts) like *Pichia* sp., *Toruspora* sp. and *Candida* sp. for winemaking (Masneuf-Pomarede *et al*., 2016). As these non-conventional yeasts may contribute to the wine's flavour and taste by producing a broad range of secondary metabolites and extracellular enzymes (Hong and Park, 2013).

Recently, new trends have emerged in wine fermentation and wine technology due to consumer preferences for wines that express their terroir with balance in acidity, mouth feels and distinguished flavour which can only be achieved through fermentation with indigenous yeasts; while some winemakers are considered about greater security and controlled variability of specialized strains (Feghali *et al*., 2020). Capozzi *et al.* (2015) reported that wines made with indigenous *Saccharomyces cerevisiae*

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Strains are perceived to show greater diversity of flavours, where these yeasts produce variable amounts of fermentative by-products, with desirable or undesirable effects on the wine.

Despite the existence of commercially modified wine yeast, there is a continuous exploration of sources for yeast species that can utilize a wide range of substrates, as it is estimated that only 1% of all yeast species have been described (Ebabhi *et al*., 2013). More so, since locally sourced yeasts (wild yeasts) and commercially available yeasts show a resemblance in their metabolic activities (Viana and Helena, 2017), it is very important to understand their respective genetic and metabolic makeup.

Banana is among the important crops grown in the tropic and the subtropic regions worldwide; highly nutritious, easy-to-digest staple food and fruit for a population of over 100 million people in Africa covering Uganda, Tanzania, Kenya, Burundi, and the Democratic Republic of Congo (Kuyu and Tola, 2018; Rossmann *et al*., 2012). Numerous strains of native yeasts have been isolated from agricultural waste like banana peels and banana pulp which is rich in carbohydrate, and other basic nutrients that support yeast growth; thus serve as readily available raw materials for the isolation of ethanologenic yeasts (Brooks, 2008; Mendonca *et al*., 2011).

Honey, an alimentary product of bees is a sugary substance (rich in glucose and fructose) gotten from the nectar of the flowers which honey bees crop, transform and store in the honeycomb of the beehive (Carvalho *et al*., 2005). Made from the nectar of numerous plant species, that have only ascomycetous yeasts; *Metschnikowia, Starmarella, Debaryomyces* and *Zygosaccharomyces* clades as the autochthonous members of the communities in the plant–bumblebee mutualism (Brysch-Herzberg, 2004).

While *Rhodotorula mucilaginosa*, *Candida magnoliae* and *Zygosaccharomyces mellis* are predominant yeast isolates in honey (Carvalho *et al*., 2010; Silva *et al*., 2017; Wen *et al*., 2017; Madden *et al*., 2018; Detry *et al*., 2020) suggesting that yeasts associated with and inhabiting tropical forests should be an important contributor to benefit our life (Deak, 2009). Thus, this study aims to characterize locally sourced yeasts from banana fruit and honey using colonial morphology, biochemical and molecular techniques and comparing them with commercially available yeasts which could be used for wine production.

Materials and Methods

Collection and Processing of Samples

Honey and wholesome ripe banana fruit were the sources from which wild yeast was isolated. The honey was obtained from Ekpoma in Edo State, Nigeria. Ripe banana fruit was bought in Fruit Garden Market, D-Line, Port Harcourt, Nigeria.

The samples were transported to the Microbiology Laboratory of Rivers State University in a sterile polyethene bag. The ripe banana was washed with clean water to remove dirt after which it was peeled for further analysis. Commercial wine yeast (CY) GV1 was obtained from the United States of America.

Isolation of Yeasts from Samples

Ten grams (10g) of the ripe banana was wholly transferred aseptically into 250 ml conical flasks containing 90 ml sterile peptone broth. Similarly, 10 ml of honey was aseptically withdrawn with the aid of a sterile 10 ml pipette from the honey stock and transferred into a 250 ml conical flask containing 90 ml sterile peptone broth. Both broths were incubated for 24-48 hours at 30 ºC for further analysis.

After incubation, an aliquot (0.1 ml) of the broth was transferred into prepared Yeast Extract Peptone Dextrose Agar (YEPDA) plates supplemented with chloramphenicol (0.003 g/L) in triplicates and was spread evenly using a sterile bent glass rod. Inoculated plates were incubated at 30ºC for 48 hours according to the method of Hong and Park (2013).

After incubation, plates were observed for growth and were subcultured on YEPDA plates for culture purification. The morphology of the yeasts was confirmed through their appearance on YEPDA plates and microscopically by viewing under the light microscope at ×100 magnification after Gram staining (Ali and Latif, 2016).

Both the wild yeast strains and the commercial wine yeasts were further identified biochemically based on their biochemical characteristics on API 20C test kits (Zaid *et al*., 2019) and molecularly, PCR and sequencing of the ribosomal ITS region (Nnodim *et al*., 2021). The commercial wine yeast was used as a reference to the isolated wild yeasts.

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Molecular Characterization of yeast isolates

The molecular characterization of the yeast isolates was carried out in the Bioinformatics Service Laboratory, Ibadan, Nigeria. The CTAB method as described by Ali and Latif (2016) and Nnodim *et al*. (2021) was used in extracting DNA from yeast strains. Accordingly, 24 hours yeast cultures in YEPD broth were centrifuged at a maximum speed of 14000 rpm for 10 minutes. Ten milligrams (10mg) of yeast cells for each strain were taken and pre-warmed in 200µl of solution I at 65°C containing 1.4M NaCl, 2% CTAB, 20mM EDTA (pH 8.0), 0.2% β-mercaptoethanol and 100mM Tris-HCl (pH 8.0) was introduced, mixed well, and incubated at 65°C for 15-20 minutes in the water bath. After incubation, all tubes were cooled for 3-5 minutes and the same volume of solution II (Chloroform: Isoamyl alcohol, 24:1) was added, mixed thoroughly, and centrifuged at 14,000 rpm for 10 minutes at 28 – 30°C. The aqueous phase (upper) was taken from each Eppendorf separately and 3M Na acetate (1/10) was introduced in each Eppendorf along with an equal volume of cold isopropanol or double volume of cold absolute ethanol, mixed it gently, and placed on ice chest for 10 minutes. All tubes after incubation were centrifuged at 12000 rpm at 4°C for 15 minutes and the supernatant was disposed. Five hundred microlitre (500µl) of chilled 70% ethanol (solution III) was added directly for washing the pellet and then centrifuged at 14000 at 4°C for 2 minutes. The pellet was air-dried after discarding the supernatant from each tube. The pellet was resuspended in 50µl double deionized water or TE-buffer and stored at −20°C. The yield of DNA was quantified by Spectro-photometer. The ribosomal DNA internal transcribed spacer region: ITS1 (GTAGGTGAA CCTGCGG) and ITS4 (TCC GCTTATTGATATGC) was used to amplify the DNA (Oliveira *et al*., 2008). The reaction mixture contained 100ng DNA, 5µl of 10pmol each oligonucleotide primer, 3µl of 25mM MgCl2, 3µl of 250mM dNTPs mixture, and Taq DNA polymerase (5 units) in a total volume of 50 µl. PCR conditions were as follows: 3 min. at 94°C followed by 35 cycles (45 sec at 94°C, 45 sec. at 55°C (annealing temperature), 1 min. at 72°C, and final extension for 7 min. at 72°C. The amplified product was determined by running on 0.8% agarose gel and visualized using a UV illuminator and photographed. More so, PCR products of the partially amplified-ITS region were subjected to restriction fragment length polymorphism (RFLP) for two restriction endonucleases TaqI and HaeIII. The reaction mixture contained 3.0µl of 1X buffer (R-buffer for BsuRI (HaeIII) and unique-buffer for TaqI), 15.0 µl PCR products (approximately 1.0 µg), 1µl of specific endonuclease, and 11µl of deionized water with a total volume of 30µl. The reaction mixtures were incubated at their specific temperatures as recommended by the manufacturer's instructions (Fermentas) The restriction fragments were separated along with a DNA 100bp ladder on 1.5% w/v agarose gel and photographed after visualization under UV light. Finally, 2.5µl of the purified PCR products were sequenced using the Applied Biosystems ABI PRISMTM 3100 DNA sequence Analyzers with the BigDye® Terminator v3.1 Cycle Sequencing kit and protocols (Shittu *et al*., 2016). The obtained DNA sequence was blasted on the NCBI gene bank to confirm the identities of the various yeasts. The following primers were used for the amplification of some functional DNA fragments by PCR: for the ATF1 Open Reading Frame (ORF), ATF1-ORF-F (caaggtaatgtgcgatcgtg) and ATF1-ORF-R (acccaagga aaatgcttgg); BAT1-ORF-F (catccaagccaagaccaaat) and BAT1-ORF-R (cacaagcagatgggtcaaga); PDC1-ORF-F (agctaacgctgctgtcccag) and PDC1-ORF-R (gtggtgaaaccaatggaacc); ARO10-ORF-F (aaccgatcagc aacaattcc) and ARO10-ORF-R (aggccagctgattca acact); ADH1-ORF-F (cggtgctgttctaaaggcc) and ADH1-ORF-R (tggacttgacgacttggttg) and, ADH2-ORF-F (tagcgcagtcgttaaggctac) and ADH2-ORF-R (gctctgttccccacgtaaga) (Parapouli *et al*., 2019).

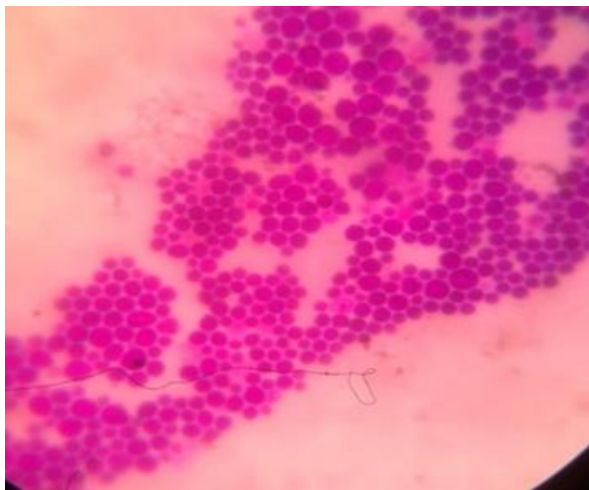
Results

The colonial morphology and the cellular morphology of the locally sourced wild yeasts and commercial yeasts are presented in Plate 1. The colonial morphology of wild yeast and commercial wine yeast possess similar characteristics. The cellular morphology of the wild yeasts as observed under the microscope were oval to round, but the commercial wine yeast cells were larger in size than the wild yeast cells.

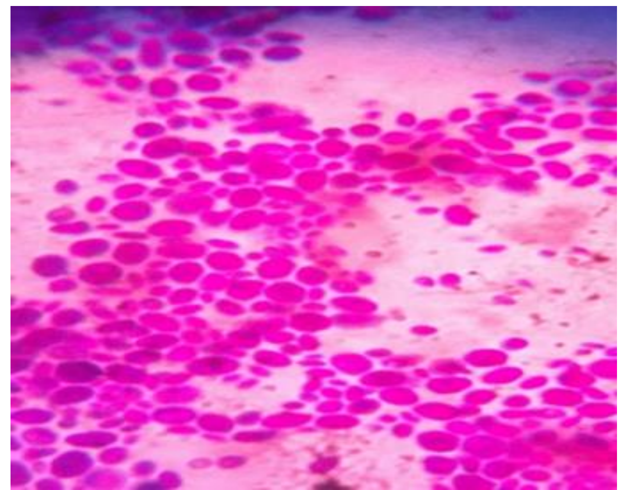
The observed reactions of yeast isolates to the various biochemical tests on the API kit are presented in Table 1, showing varied responses by the yeast isolates to the reagents. The API kit test result shows that galactose, glucose, and sucrose were fermented most especially glucose and sucrose which are the major sugars found in must. On the other hand, erythritol and melibiose are not fermented by both the wild yeasts and commercial wine yeast. Based on the fermentative activities of the isolates B^{10}, B^{7}, H^{4}, and H^{7} on galactose, glucose and sucrose, they were considered effective and efficient fermenters for wine production using CY as a yardstick or control.

1a: Colonial morphology of locally sourced wild yeast

1b: Colonial morphology of commercial wine yeast GV1



1c: Cellular morphology of locally sourced wild yeast



1d: Cellular morphology of commercial wine yeast GV 1

Plate 1: Colonial and Cellular morphology of wild yeast and commercial wine yeast

The result of the local BLAST of the biochemical response of the yeast isolates is presented in Table 2. The local BLAST of the biochemical response of the yeast isolates using API/ID 32C V2.0 kit confirmed the isolates B^7, B^{10}, H^4, H^7, and CY with 99.90% relatednesses to *Saccharomyces cerevisiae, Candida tropicalis, Candida hellenica, Saccharomyces cerevisiae,* and *Candida kefyr* respectively (Table 2).

The phylogenic tree showing the evolutionary distance and evolutionary history of the five (5) yeast isolates as inferred from their amino acid sequence using the Neighbour-Joining method and evolutionary distances using the Poisson correction method is presented in Figure 1.

The unrooted phylogram grouped the yeast isolates into two (2) clades, with the rate of mutation per amino acid sequence site of 0.103 and 0.365 for the first clade and second clade respectively. The first clade consists of *Candida tropicalis*^{H4}, *Candida tropicalis*^{B10}, and *Candida tropicalis*^{CY} being a paraphyletic taxon. *Candida tropicalis*^{H4} and *Candida tropicalis*^{B10} are 100 % similar in amino acid sequence, but with 0.10 and 0.02 mutations per sequence site, respectively, while *Candida tropicalis*^{CY} has 1.007 mutations per sequence site. The second clade consists of *Candida tropicalis*^{B7} and *Clavispora lusitaniae*^{H7} with 86 % similarity in amino acid sequence and mutation per sequence site of 1.11 and 0.00, respectively.

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Table 1: Biochemical Test Result of Yeast Isolated From Banana, Honey and the Commercial Yeast Using API/ID 32C V2.0

Isolate	Galactose	Actidione	D-saccharose	NAG	Lactic acid	L-Arabinose	D-Cellobiose	D-Raffinose	D-Maltose	D-Trehalose	2KG	MDG	D-Mannitol	D-Lactose	Inositol	D-Sorbitol	D-Xylose	D-Ribose	Glycerol	L-Rhamnose	Erythritol	D-Mellibiose	GRT	D-Melezitose	GNT	Levulinic acid	D-Glucose	L-Sorbose	Glucosamine	Esculin	Palatinose
B[7]	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
B[10]	+	+	+	+	+	-	+	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	+	-	+	+	+
H[4]	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	+
H[7]	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
CY	+	+	+	-	+	+	-	+	-	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	+	-

Keys: B= yeast isolated from banana, H= yeast isolated from honey, CY= commercial wine yeast**;** NAG = N-acetylglucosamine; 2KG = 2-keto-D-gluconate; MDG = α-Methyl-D-glucoside; GRT = Sodium glucuronate; GNT = Potassium gluconate

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Table 2: Identification using Analytical Profile Index (API)/ID 32C V2.0 kit local BLAST Hits and Their Percentage Relatedness

Isolate code	API BLAST relative	Percentage (%) ID
B7	*Saccharomyces cerevisiae*	99.90
B10	*Candida tropicalis*	99.90
CY	*Candida kefyr*	99.90
H4	*Candida hellenica*	99.90
H7	*Saccharomyces cerevisiae*	99.90

Key: B= yeast isolated from banana, H= yeast isolated from honey, CY= commercial wine yeast.

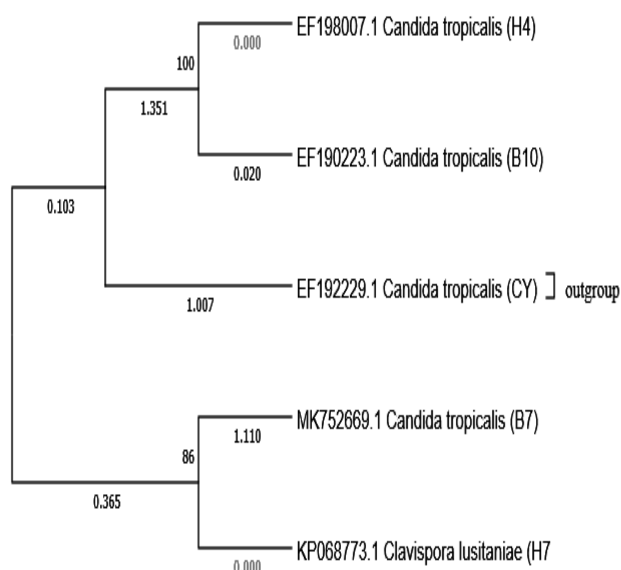

Fig. 1: Phylogenic tree of evolutionary distance and evolutionary history of the five yeast isolates

The results of the molecular characterization of the yeast isolates revealed that yeast isolates and their NCBI BLASTN Relative and relatedness (%) is shown in Table 3. The results of the molecular characterization of the yeast isolates revealed that yeast isolates B^7, B^{10}, H^4, H^7 and CY with accession numbers MK752669, EF190223, EF198007, KF268353 and EF192229 had 93.10%, 95.00%, 94.80%, 77.60% and 92.10% relatedness to *Candida tropicalis* strain Pe 1, *Candida tropicalis* strain WC65-1, *Candida tropicalis* strain WC57, *Clavispora lusitaniae* strain WM03 and *Candida tropicalis* strain zhuan4 respectively.

Functional gene analysis

The screening results for some essential functional genes associated with wine yeast in the wild yeast isolates are shown in Plates 3 - 8. The wild yeasts possess Alcohol O-acetyltransferase (ATF) 1 gene, Pyruvate decarboxylase (PDC) 1 gene, Branched-chain-amino acid transaminase (BAT) 1 gene, Thiamine pyrophosphate-dependent 2-oxo-acid decarboxylase (ARO) 10 gene, and Alcohol dehydrogenase (ADH) 1 and 2 genes in their chromosomal DNA. The size of the various functional genes in the yeast isolates ranges from 140 – 200 (bp). The yeast isolates (B^7, B^{10}, H^4, H^7, and CY) possess Alcohol O-acetyltransferase (ATF) 1 gene (170bp), Pyruvate decarboxylase (PDC) 1 gene (200bp), Branched-chain-Amino acid Transaminase (BAT) 1 gene (150bp), Thiamine Pyrophosphate-dependent 2-oxo-acid Decarboxylase (ARO) 10 gene (140bp) and Alcohol Dehydrogenase (ADH) 1 and 2 genes (180bp and 200bp) gene in their chromosomal DNA respectively, except isolate B10 that lack PDC 1 and ARO 10 genes in their chromosomal DNA.

Table 3: Sequence Identification from National Center for Biotechnology Information (NCBI) blast hits and their percentage relatedness

Isolate code	NCBI BLASTN Relative	Accession Number	E Value	% Relatedness
B7	*Candida tropicalis* Pe 1	MK752669	2.50E-179	93.10
B10	*Candida tropicalis* WC65-1	EF190223	0	95.00
CY	*Candida tropicalis* zhuan4	EF192229	0	92.10
H4	*Candida tropicalis* WC57	EF198007	0	94.80
H7	*Clavispora lusitaniae* WM03	KF268353	8.52E-35	77.60

Key: yeast isolated from banana, H= yeast isolated from honey, CY= commercial wine yeast

Citation: Nnodim and Odu. (2023). Characterization of wild yeasts isolated from banana fruit and honey for wine production. *International Journal of Microbiology and Applied Sciences*. 2(2): 11 - 23.

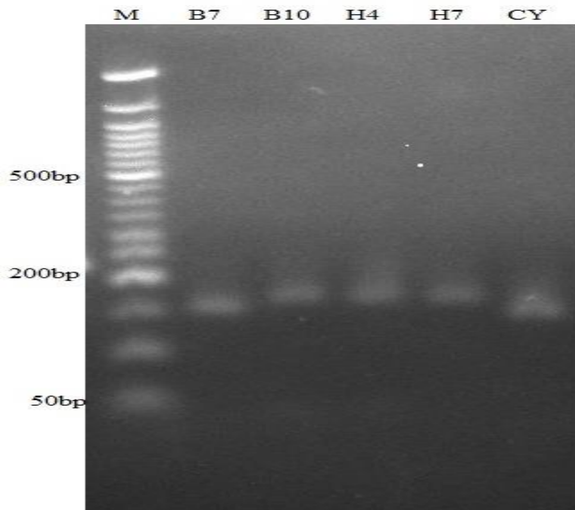

Plate 3: Alcohol O-acetyltransferase (ATF) 1 gene

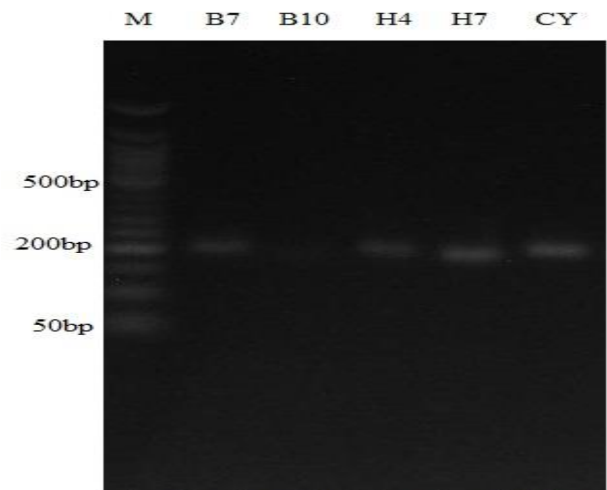

Plate 4: Pyruvate decarboxylase (PDC) 1 gene

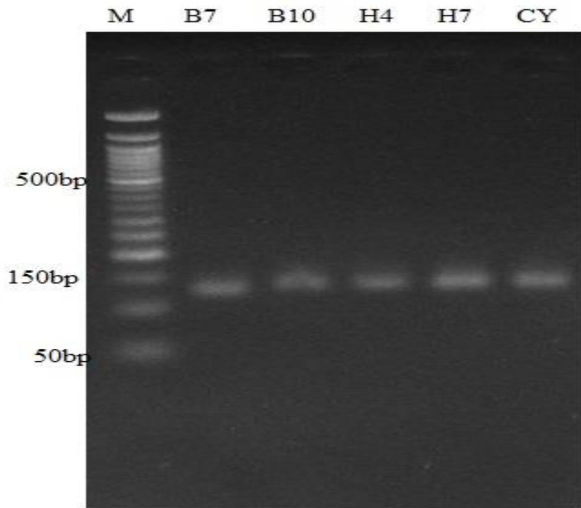

Plate 5: Branched-chain-amino acid transaminase (BAT) 1 gene

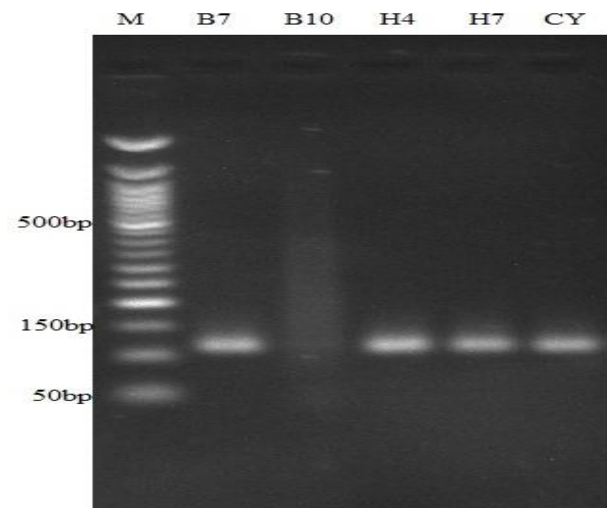

Plate 6: Thiamine pyrophosphate-dependent 2-oxo-acid decarboxylase (ARO) 10 gene

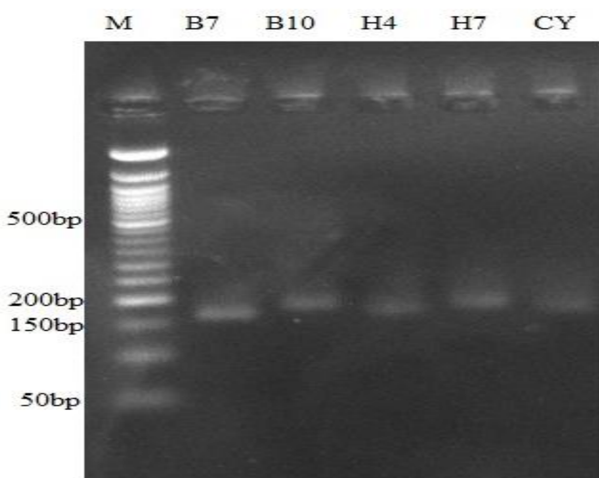

Plate 7: Alcohol dehydrogenase (ADH) 1 gene

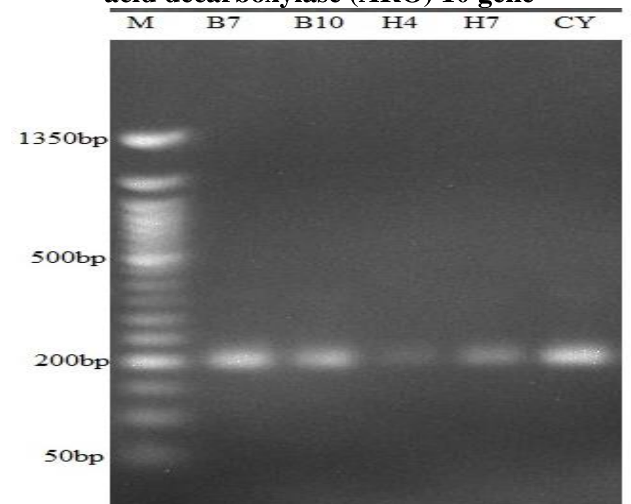

Plate 8: Alcohol dehydrogenase (ADH) 2 gene

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Discussion

The increased consumer preference for wines that meets their terroir as reported in previous studies (Marlowe and Bauman, 2019; Vilela, 2019), does not only depend on the substrate but, also on the type of yeast strain that ferments the substrates other than grapes and *Saccharomyces cerevisiae* to yield the desired wine (Kumar *et al*., 2017; Swaroop *et al*., 2017). Isolation of yeast from banana fruit and honey was in agreement with the report of Vegas *et al*. (2020) that fruits establish outstanding habitats for yeasts, due to their low pH, nutrients, sugar availability, and dynamic fruit-associated vectors. Beyene *et al*. (2020) also stated that Tej (honey wine) an indigenous fermented drink has yeasts as predominant fermenters. Ramírez-Castrillón *et al*. (2019) reported that fruits are stimulating substrates for the growth of yeast species because fruits are composed of water and carbohydrates, which offer a perfect environment for growth. The yeast isolates exhibited creamy, raised, dull and smooth surfaces, smooth edges and large colonies, which is in agreement with previous reports that yeasts can create organized colonies that range from oval cells to filamentous cell shapes (Palková and Váchová, 2016), white or cream colouration, entire margin, smooth surface, raised elevation and dome-like, or conical shape (Kurtzman *et al*., 2011). It was observed under the microscope that cells of commercial wine yeast were large in size, this could be because the lifestyle of wild and laboratory yeast strains significantly differs in ability to form extracellular matrix that influences colony architecture among distinct strains, depending on their particular genetic background (Šťovíček *et al*., 2010).

Commercial wine yeast (CY) served as a reference to the isolated wild yeasts in understanding their phylogenic relationship and fermentative abilities. The biochemical tests showed that all the wild yeasts like the commercial yeast fermented D-saccharose, D-Glucose, galatose and responded differently to other substrates. The results of this study are in agreement with the report of Tan-Gana *et al*. (2014) that there is variation in fermentation ability among yeast strains; thus. confirming the biodiversity of yeasts within our environment (Ayanniran *et al*., 2020). Commercial wine yeasts and wild yeasts employed in industrial works can exhibit great differences, both in genetic and physiological characterization even when of the same species (Camargo *et al*., 2018).

All the yeast isolates were able to assimilate glucose, fructose and sucrose (Camargo *et al*., 2018) and they were mainly of two genera, *Saccharomyces* sp. and *Candida* sp. (Sulieman *et al*., 2015). But the technique is not very accurate as some of the expressed biochemical or metabolic response could be controlled by a particular gene, and sometimes there are variations in the physiological response (Fernández-espinar *et al*., 2011).

Recently, characterization of yeasts has adopted techniques based on phylogenic relationships brought about by gene sequencing, and this technique which considers the DNA-DNA hybridization of the isolates, electrophoretic karyotyping, and polymerase chain reaction is regarded as molecular classification or characterization (Ali and Latif, 2016; Fernández-espinar *et al*., 2011). The variation in the strain of *C. tropicalis* is also reflected in the fluctuations in physiological (biochemical) response. The use of this method in classifying strains of *S. cerevisiae* is known to be very efficient since the addition or elimination of long fragments of DNA in homologous chromosomes during the evolution of the yeast genome is shown in the polymorphism (Fernández-Espina *et al*., 2011). More so, Ali and Latif (2016) reported that characterizing yeast strains isolated from different sources using Restriction Fragment Length Polymorphism, a molecular-based method identified different strains. The *C. tropicalis* in other studies had 400bp bands as those of the present study, but *Clavispora* spp was found in the 800bp which disagrees with the placement in this study in which *Clavispora lusitaniae* WM03 had 650bp.

The prominent microorganisms used in wine production that through different activities determine the qualities and flavour of wines are the yeasts. Although *S. cerevisiae* which has raised speculations and controversies as to its origin since most researchers have not been able to isolate it from healthy grapes is still the main species in wine production, other species play important roles. More recently, winemakers and wine researchers have come to realize that non-Saccharomyces yeasts also contribute to the flavour and quality of wine to a greater extent than previously thought (González *et al*., 2007).

The desired wines can be achieved when yeasts possess stress tolerance and the ability to completely metabolize sugar content in a substrate.

Thus, screening of wild yeasts for certain fermentative characteristics, and understanding their phylogeny and genetic make-up would be an important tool to consider in wine production, as it enables oenologists to select yeasts that are best suited. This is in consonance report of Feghali *et al*. (2020) that certain criteria are desired to guarantee a yeast strain's ability for winemaking.

This present study has shown that the wild yeast isolates locally sourced possess the functional genes or molecular characteristics that make them fit to be employed for wine-making. Alcohol O-acetyltransferase I (ATF1) gene was referred to as a gene of oenological relevance (van Wyk *et al*., 2020) that is inherent for esters or higher alcohols synthesis for flavour production in fermented alcoholic beverages (Holt *et al*., 2018; Hong *et al*., 2019), which is responsible for the fruity character of fermented alcoholic beverages and comparatively produced at a higher concentration by non-Saccharomyces species (Moon *et al*., 2021). Borodina and Nielsen (2014) and Gancedo *et al*. (2016) reported that pyruvate developed in glycolysis is localized at fermentative metabolism by Pyruvate decarboxylase (PDC); accounting for about 90 % of the proteins and controlled by available carbon. Agarwal *et al*. (2015) also stated that *Saccharomyces cerevisiae* produces acetaldehyde through fermentative decarboxylation of pyruvate, thus, PDC decarboxylation activity is indispensable, as the PDC1 gene is involved in substrate recognition and activation, catalysis, and stability. Milanovic *et al*. (2012) acknowledged that the PDC1 gene was also expressed by non-Saccharomyces.

The place of the Branched-chain Amino acid Transaminases (BAT)1 gene in quality alcoholic beverage production was confirmed by van Wyk *et al*. (2019) and the lack of BAT 1 gene in *Hanseniaspora vineae* reduced the production of branched-chain higher alcohols, fatty acids, and ethyl esters (Belda *et al*., 2017; Giorello *et al*., 2018), indicating that BAT 1 enzymes are responsible for ester synthesis in non-Saccharomyces yeast (Belda *et al*., 2017) and rate of microbial growth (Lilly *et al*., 2006). *Saccharomyces cerevisiae* ARO10 gene encodes phenylpyruvate decarboxylase activity, which is involved in the Ehrlich pathway for amino acid metabolism (Deed *et al*., 2019; Valera *et al*., 2020). In the Ehrlich pathway, the α-keto acid is typically decarboxylated to an aldehyde is reduced to higher alcohols by alcohol dehydrogenase enzymes (Deed *et al*., 2019) and overexpressed ARO10 results in 8-fold higher isobutanol production as compared to the control strain in micro-aerobic fermentation according to Li *et al*. (2017) and overexpression of the ARO10 gene increased 2-Phenylethanol production from glucose (Shen *et al*., 2016; Yin *et al*., 2015). Alcohol Dehydrogenase (ADH) is an enzyme that reduces acetaldehyde to ethanol during the fermentation of glucose (Raj *et al*., 2014), and impairing ADH expression and activity brings about low ethanol production as it plays an important role in yeast fermentation (Varela *et al*., 2012). Suwannarangsee (2010) illustrated that optimization of ADH gene expression is an ideal approach for developing an efficient bioethanol production yeast strain (Wang *et al*., 2009).

In conclusion, this study has established that ripe banana fruit and honey are local sources that harbour yeast of biotechnological importance. Of some of the locally sourced wild yeast isolates possess ATF1, PDC1, BAT1, ARO10, ADH1 and ADH2 genes of which expression is essential for quality winemaking. The functional genes isolated from wild yeast are also associated with commercial wine yeast and the molecular weight of the functional genes in both wild and commercial wine yeast range from 400 – 600 base pair.

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