Molecular Phylogeny of Yeasts from Palm Wine and Enological Potentials of the Drink

Ogueri Nwaiwu¹* and Martin Itumoh²

¹Alpha Altis, Ingenuity Center, University of Nottingham Innovation Park, Triumph Road, Nottingham, NG7 2TU, United Kingdom.
²Department of Agribusiness and Management, Faculty of Agriculture, Federal University Ndufu Alike Ikwo, Ebonyi State, Nigeria.

Authors’ contributions

This work was carried out in collaboration between both authors. Author ON designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author MI managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/37748
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Moutawakilou Gomina, University of Parakou, Benin.
(2) Hatice Kalkan Yildirim, Ege University, Turkey.
(3) Angelo Maria Giuffrè, Università degli Studi Mediterranea di Reggio Calabria, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history/22091

Received 27th October 2017
Accepted 25th November 2017
Published 29th November 2017

ABSTRACT

Palm wine is consumed around the world and known by various names. Unlike many good quality traditional fermented food and drink in developing countries that have only been marketed in their local communities, palm wine has proved to be a commercial success in that it is now canned or bottled and marketed internationally. Despite these advances, the drink has not been subjected to current technology applied to wines from grapes to improve quality. This review highlights the evolutionary relationships between yeasts isolated from the drink in two continents and the enological potential of the drink. The evolutionary history was inferred by using the Molecular Evolutionary Genetics Analysis (version 7) computer software to construct phylogenetic trees using 34 previously reported curated nucleotide sequences from the D1/D2 domain of 26S rRNA in yeasts isolated from the drink. We found that similar yeast species formed terminal taxa from a common node. However Pichia kudriavzevii species formed two distinct clades which suggest intra-species diversity. One Saccharomyces cerevisiae (accession No. HG425326) had a node distinct from other

*Corresponding author: E-mail: ogueri.nwaiwu@alpha-altis.co.uk;
1. INTRODUCTION

Palm wine is a drink consumed in many parts of the world and it is known as a global fermented beverage [1]. It is obtained from palm saps of different palm trees and consumption is common in South America, Africa and Asia. What was originally a drink for the poor is now canned, bottled and marketed internationally. The drink is consumed by millions of people in West Africa even though the nutritional aspects of the drink is still largely unknown [2]. Apart from direct consumption, palm wine can be distilled to produce a local gin with ethanol content that can range from 20% [3] to 61.5% v/v [4]. At the start of palm wine fermentation, common bacteria found in the drink includes lactobacillus whereas acetic acid bacteria (AAB) are found at the end [5]. The yeast Saccharomyces cerevisiae ferments sugar in the middle of palm wine fermentation to yield metabolites like ethanol.

In the last 10 years, the use of molecular techniques (26S rDNA sequencing) for identification has revealed the actual yeast species inherent in palm wine and the general consensus is that Saccharomyces yeasts especially S. cerevisiae is the dominant yeast among other naturally occurring microbial flora of the drink. However, it is now believed that non-Saccharomyces yeasts may have an impact on volatile composition and sensory profile of fermented beverages [6,7]. New palm wine yeast species like Hanseniaspora jakobsenii [8] and Clavispora lusitaniae [9] have been identified from the drink and there could be potential for more yeast species discoveries since the molecular characterization of microorganisms present in the drink has not been fully explored around the world to explain species diversity. It is not yet clear how the yeasts that ferment palm wine evolved. From literature, it is evident that palm wine yeast content varies among the various regions of the world. A study in Mexico [10] failed to produce S. cerevisiae as the dominant yeast in a palm wine sample and in an earlier study in Nigeria it was suggested that S. cerevisiae population is autochthonous [11].

However, another study [12] showed that some S. cerevisiae strains of Nigerian palm wine are similar to well characterized reference strains (NCTC 1406 and strain S288c) used around the world. In Burkina Faso investigators found that B. akeassii palm wine contains a specific yeast population of diploid strains different from other palm wine strains from Ivory Coast, Nigeria and Djibouti Republic [13].

The palm wine production process has been explained in a previous report [12] and varies from one continent to another. A study by Legras et al. [14] investigated how the yeast species S. cerevisiae evolved. The scientists in that study analyzed the genetic diversity among 651 strains from 56 different geographical origins worldwide and their genotyping at 12 microsatellite loci revealed genotypes that were organized in subgroups in popular products consumed around the world namely bread, beer, wine and sake. It was found that some of these groups displayed a combination of alleles that were intermediate between these subgroups and strains genetic diversity was linked with geographical differences which suggested local domestications.

There has been increase in the last 5 years on identification of microorganisms that ferment palm wine, but not much has been done to identify all the secondary metabolites of bacteria and yeasts that make up the flavor and aroma of the drink. In particular, there are scarcely any detailed study that has used current biotechnology analytical tools to elucidate the interactions between bacteria and Saccharomyces or non- Saccharomyces yeasts in flavor or aroma generation. Production of the drink is still small scale and in order to make larger volumes and improve the availability of the drink, there is a need to adopt studies and methods that have been used to gain more understanding of the fermentation chemistry of different well known wines and spirits made from fermentation of other food products especially

\[ S. \textit{cerevisiae} \] species indicating that the strain may belong to a different lineage. From literature, the enological compounds measured in different types of wines like ethyl acetate, oleic acid, ethanol, and acetic acid are present in palm wine. These primary and secondary metabolites of palm wine from different yeasts confirm the enological potentials of the drink even though higher alcohols and esters appear to be more in some wines when compared to palm wine. Future research may involve more holistic investigations that will elucidate the biochemical pathway of palm wine fermentation products.

Keywords: Phylogeny; evolution; enology; metabolites; palm wine; yeasts.
grapes. This approach may include gene manipulation and full metabolomics assessment.

The difference between the end products of fermentation of wine made from grapes and palm wine obtained from palm sap is that the former undergoes several processes to improve quality whereas the latter is taken in the form obtained from the field. Both types of wine are believed to contain beneficial anti-oxidants like phenols. The level varies during wine production [15]. The actual level of benefits obtained from antioxidants in wine is still debated. It has been reported that red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans [16] while another study pointed out that antioxidants like resveratrol in red wines in low doses, has no beneficial effect [17]. The antioxidant profile of palm wine is not well studied. However, a study on humans that consume the drink regularly revealed that consumption of the drink may deplete the body’s antioxidants against free radical attacks and render the body in a state of oxidative stress [18]. Generally, epidemiological evidence suggests that the potential health benefits of phytochemical constituents in grapes include protection against oxidative damage, anti-diabetic, anti-cholesterol, and anti-platelet functions [19,20].

The outline by Walker and Stewart [21] shows that after fermentation of sugars by S. cerevisiae in fermented beverages, further distillation of the fermented mix can be performed to produce matured spirits like whiskey or non-matured spirits like gin. Currently palm wine is only distilled into non-matured spirits. The maturing of the drink and its distillate by fortification with other microorganisms is yet to be explored commercially. The application of metabolic engineering of S. cerevisiae to palm wine production has not been performed. It is worth exploring because the organism is known as an effective cell factory for metabolite production [22].

Previous studies carried out on palm wine volatiles using GC-MS [23] and proton transfer reaction-mass spectrometry [24] show that palm wine is a reservoir of several flavor and aroma volatiles found in well-known red and white wines [25] and other formulated wines [26]. Common groups of flavor and aroma volatiles found in palm wine by the aforementioned investigators include alcohols, carboxylic acids, phenols and esters. These compounds have been confirmed by other palm wine investigators [12,27,28].

Many traditional foods have been reported to be interesting and useful as food and for human health but no extensive scientific analysis has been carried out on them possibly because they lack a commercial potential. The case of palm wine is different in that the drink is now canned or bottled and sold internationally. Therefore, more studies are required for a better understanding of the nature of metabolites from yeasts in palm wine and how the organisms evolved. Microbial research on foods enabled technological developments in Japan in the past [29]. Therefore, more studies may lead to application of current biotechnology analytical methods for further research in flavors of the drink and may result in sustained production of improved varieties of palm trees and post-harvest fermentation of palm wine with different flavors. Also, it has been pointed out that traditional food fermentation for products like palm wine represents an extremely valuable cultural heritage and may harbor undiscovered strains [1]. It was emphasized that holistic approaches for identification are of interest to food microbiologists.

The phylogeny of palm wine yeasts using molecular characteristics has not been well reported in literature. This review summarizes the phylogenetic relationships of curated yeasts isolated from palm wine from two continents and highlights the winemaking or enological potentials of the drink.

2. PALM WINE YEAST PHYLOGENY

In order to elucidate the relationships between palm wine yeasts from West Africa and South America, phylogenetic analysis was carried out. We used curated yeast sequences covering the D1/D2 domain of the 26S rRNA of yeast strains obtained from previous reports. This included sequences from reports of palm wine produced in Burkina Faso [5] and Nigeria [12] both in West Africa and Mexico, South America [10]. First, a multiple sequence alignment (MSA) was constructed with the curated sequences mentioned above by using multiple sequence comparison by log expectation (MUSCLE) described by Edgar [30] after which five phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA; version 7) tool developed by Kumar et al. [31]. The MSA tool MUSCLE was used
because it is rated among the top 10 widely used and has been shown to produce very good alignments [32,33].

To determine strains close to wine yeasts, one control sequence (Accession No. JX423566.1) which was reported [12] to have a 100% identity match with a wine yeast was included. A sequence found in the bacteria strain *Gluconacetobacter saccharivorans* (Accession No. HE979557) isolated from palm wine [5] was also added as an out group. The statistical methods used for constructing the trees included neighbor joining, minimum evolution, maximum parsimony, unweighted pair group method with arithmetic mean (UPGMA), and maximum likelihood. Evolutionary distances were computed using the maximum composite likelihood model and test of reliability was carried out by bootstrapping [34] with 1000 replicates in all cases. The neighbor joining and minimum evolution methods had the same topology after phylogenetic trees construction and all the 5 trees constructed showed virtually the same monophyletic groups. For brevity, the UPGMA (Fig. 1) is shown. The tree expectedly showed a different root source for the bacteria species used as an out-group.

The yeast species containing the *S. cerevisiae* positive control sequence (wine yeast) originated from the same node as other *S. cerevisiae* species. Overall, similar yeast species formed terminal taxa from a common node. However, *Pichia kudriavzevii* species formed two distinct clades which suggest intra-species diversity. In particular, *P. kudriavzevii* (accession No. HG425333) from Nigeria formed a separate clade with a strain (accession No. KF241564) from Mexico. This needs further investigation to ascertain how they differ with other strains of *P. kudriavzevii*. One *S. cerevisiae* (accession No. HG425326) had a node distinct from other *S. cerevisiae* species indicating that the strain may belong to a different lineage. Intra-species diversity of other palm wine microorganisms has been reported [5,13] and the bootstrap values suggest that intra-species diversity may be more in *S. cerevisiae* species than other yeast genera like *Pichia* and *Candida*.

3. ENOLOGICAL QUALITIES OF PALM WINE: COMPOUNDS OF PALM WINE FOUND IN WINES

In most cases unless otherwise stated the term ‘wine’ is applied to product of fermentation of grape juice by yeast [35]. Metabolites produced during wine fermentation may be primary or secondary. The primary metabolites are involved in growth whereas secondary metabolites are not important to maintain cell life. The profile knowledge of metabolites is essential for functional genomics and search for new compounds that may be used as biotechnology products [36]. The production of primary compounds which includes ethanol, glycerol, acetic acid and secondary metabolites like fatty acids, esters, and higher alcohols contributes hugely to the flavor and aroma of wines [37]. The chemical components usually found in grape wines have been reported to be present in palm wine. In enology, *S. cerevisiae* is the yeast mainly used [38] and it is commonly found in palm wine. According to a study with 70 different strains, it was found that the organism appeared to enhance antioxidant and phenolic compounds content in red wines used for that study [39].

It has been reported that *S. cerevisiae* can be manipulated genetically to give a desired product [40]. However, there is evidence that non-*Saccharomyces* yeasts like *Pichia* sp. found in grapes [41] and palm wine [42] can produce other compounds like glycerol rather than alcohol after sugar fermentation [43] and improve the flavor of wines. Specific non-*Saccharomyces* yeasts highlight how vital strain selection is, because these organisms can secret enzymes and also produce secondary metabolites which aid stability and color in wine [44]. The common chemical compound classes [26] that can be found in wines are listed in Table 1. These compounds (Nos 1-6) are also found in palm wine except C13-Norisoprenoids and terpenes which are not widely reported in palm wine possibly because they are found mainly in grape skin [45]. The occurrence of terpenes in palm wine in the studies of Uzochukwu et al. [23] and Lasekan [24] may be due to environmental contamination from palm or wild fruits. The four most common chemical groups found in palm wine include ethyl acetate, oleic acid, ethanol, and acetic acid. It appears that the number of esters can vary in palm saps because a Nigerian [23] palm sap (E, Table 1) had more esters than the Canadian [26] wine (A, Table 1) and Malaysian [28] palm sap (C, Table 1).

3.1 Ethyl Acetate

In most palm wine studies, ethyl acetate is one of the esters that are dominant. Esters are highly
valued in wine making because of the sweet smelling aroma it brings to wines and beer [46]. Among other wines, ethyl acetate is found in red [47] white [48] and durian wines [49]. It is also a key aroma compound in apple [50] and bilberry syrup wines [51]. Fermentation with brewer’s yeast has shown that esters are formed intracellular after catalysis by an acyl transferase [52]. The genes that encode synthesis of acetate ester like ethyl acetate are well characterized and the biochemical pathways of the compound synthesis are well studied [53]. This knowledge can be applied to palm wine fermentation to generate higher ester concentrations.

Fig. 1. Evolutionary history of yeast strains with verified sequences covering the D1/D2 domain of the 26S rRNA from previous reports using the UPGMA method. Species with accession numbers beginning with HG obtained from Nigeria [12], HE- from Burkina Faso [5] and KF from Mexico [10] were included. The bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, and the analysis was carried out with 34 nucleotide sequences
Table 1. Major chemical compound classes of wine from Canada (A) compared with volatiles from palm wine reported in four different studies (B – E)

<table>
<thead>
<tr>
<th>Wine volatiles</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Esters</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>2. Fatty Acids</td>
<td>2</td>
<td>2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>3. Alcohols</td>
<td>NS</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4. Carboxylic Acids</td>
<td>NS</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>5. Acyloins</td>
<td>NS</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6. Phenols</td>
<td>2</td>
<td>1</td>
<td>NS**</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>7. C13-Norisoprenoids</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8. Terpenes</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9. Other Products*</td>
<td>6</td>
<td>NS</td>
<td>1</td>
<td>23</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Compounds that appeared to be derivatives were grouped into volatiles of Other Products;
**NS = not stated; N/A = not applicable

A = Canadian wine study [26]; palm reports: B [12], C [28], D [27] and E [23]

3.2 Oleic Acid

Oleic acid is found in the cytoplasm of *S. cerevisiae* and is listed as the most widely distributed and abundant fatty acid in nature [54]. It has been reported that ethanol tolerance in *S. cerevisiae* is dependent on cellular oleic acid content [55]. Three strains of *S. cerevisiae* have been analysed during hypoxic growth in a sugar-rich medium lacking lipid nutrients and it was found that lack of lipids may have a negative effect on yeast viability [56]. This analysis was confirmed by Landolfo et al. [57] after it was shown that lipid nutrients facilitate a reduction of intracellular oxidative damage to membranes and proteins. Another factor that could affect oleic acid abundance is temperature. It has been demonstrated that temperature can affect the lipid composition of *S. cerevisiae* and *S. kudriavzevii* during wine fermentation [58]. Palm wine is currently not processed under cold storage and the effects of temperature or cold storage processing on flavor and aroma is not known.

3.3 Ethanol and Phenols

Ethanol is the alcohol that is easily detected in wines and palm wine. The distinct aroma is recognizable by most people and the compound varies in concentration of wines and palm wine from different regions of the world. Yeasts resistance to ethanol in palm wine and wines is generally recognized to be the reason the organism dominates during beverage fermentation. This has been attributed in part to yeast's physiological characteristics which were not modified throughout the adaptation to human-manipulated fermentative environments [59]. Wines contain more ethanol than palm wine probably due to the controlled manner production is carried out with established standards. Polyphenolic antioxidants are believed to be beneficial to health and are present in wines [60]. Phenolic compounds are believed to be antifungal, antiviral and anti-bacteria [61]. Phenolic content may depend on the wine origin [62]. The characteristics of phenols from palm wine are unknown and needs to be investigated.

3.4 Carboxylic Acids and Acyloins

Acetic acid bacteria (AAB) can cause spoilage and affect the quality of wine [63]. They originate from infected grapes [64] and the main carboxylic acid formed is acetic acid [65]. In palm wine processing, AAB is responsible for the vinegar taste due to production of acetic acid at the end of fermentation and a previous characterization [5] showed a predominance of *Acetobacter* species. In that characterization, a high level of diversity was observed at the intra-species level and it was suggested that *Acetobacter* species may have a determinant role in the later stages of palm wine fermentation. Acetoin belongs to the compound class acyloins which are organic compounds containing an alpha hydroxy ketone. It has a pleasant buttery odor and is a component of the butanediol cycle in microorganisms where it is used in the cytoplasm of yeasts as an external energy store [54]. The compound appears to have an aroma function in wines and palm wine.

3.5 Quantity of Alcohols and Esters in Palm Wine and Standard Wines

According to Stribny et al. [66], acetate esters and higher alcohols produced during
fermentation by yeast significantly contributes to the aroma character of alcoholic beverages. The report highlighted that the major component of aromatic higher alcohols is 2-phenylethanol that can be generated from aromatic amino acid phenylalanine [67]. A comparison of the palm wine samples from a previous report [12] with the alcohol and ester content of some wines described by Cheng et al. [68] showed that the higher and primary alcohols were generally lower in various palm wine samples (Fig. 2). The Italian Riesling wine had the highest amount of 2-phenylethanol and ethyl acetate whereas 1-propanol and ethyl octanoate occurred highest in cabernet sauvignon wine. The wine processing method used may have caused these differences. Also the generation of alcohols and esters can be affected by pH, temperature, substrate concentration [69] and fermentation medium gas-liquid balance [70]. Furthermore, the yeast strains used for fermentation may determine the flavor compounds produced [71].

4. PROSPECTS OF VINTAGE PALM WINE

A previous investigation explored the possibilities of fermenting palm wine in a controlled environment [72] by using a model that compared well with experimental results. Some other studies have been carried out by using yeasts from Nigerian palm wine to make beer [73] and fruit wines from mango [74], pine apple [75] and water melon [76] with promising results. However, these efforts in the laboratory have not been translated to commercial success even though the technology to make this possible is now available. It has been shown that industrial yeasts can be bred for direct commercial application [77] and the ATF1 genes responsible for sweet smelling esters in *Saccharomyces* species [78] can be altered to make new or improved flavors. This can be applied to palm wine production by selection of novel strains for better aroma and flavor. The authors are not aware of any country that has approved the use of genetically modified (GM) yeasts for palm wine production. However, application of GM yeast to palm wine production may be commercially relevant in that the fermentation period of palm wine may be extended or delayed to produce a desired flavor.

A previous report [79] has shown that production of primary and secondary metabolites is complex and requires creative designs to develop new technology. These new methods in addition to enological parameters optimization has enabled standardization of fermented foods in developed countries [80]. Yang et al. [81] has suggested that if traditional fermented foods are promoted with high throughput biotechnologies, the industrialization of local fermented food will increase.

![Fig. 2. Comparison of some alcohols and esters content in palm wine (Pw) samples from previous reports. Samples from two different types of trees [12] namely Elaeis sp. (E) and Raphia sp. (R) were compared with different types of wine [68]](image)
5. CONCLUSIONS

The phylogenetic analysis showed intra-species diversity in *Pichia kudriavzevii*. This needs to be taken into consideration when the roles of specific yeast species from different continents are investigated for aroma or flavor production. The enological compounds found in different types of wines are present in palm wine but it appears that some esters and the key higher alcohol (2-phenylethanol) occur in greater amounts in classic red and white wines when compared to palm wine. Now that palm wine is canned and sold internationally, increasing commercial demand may result in holistic application of modern analytical tools to develop new quality standards for the drink. Increased research that will follow in future may help determine the best candidate yeast strains for fermentation of the drink across the world. Yeast selection will largely depend on the flavor or aroma compounds produced.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


76. Okeke BC, Agu KC, Uba PO, Awah NS, Anaukwu CG, Archibong, et al. Wine production from mixed fruits (pineapple and watermelon) using high alcohol


