Chapter 2

Phylogeny of Three Palmwine Yeasts Genera

Ogueri Nwaiwu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79958

Abstract

Sequences from three palm wine yeast genera namely *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, and *Candida ethanolica* were analyzed to establish their phylogenetic relationships, geographical origin, and food matrix source of their close relatives. Up to 600 sequences present in yeasts representing close relatives of palm wine yeasts were examined. Pyhlogenetic trees constructed showed polyphyletic relationships in *C. ethanolica* whereas close relatives of *S. cerevisiae* and *P. kudriavzevii* showed little divergence. Sequence data for both *Elaeis* sp. and *Raphia* sp. palm trees showed that highest number of palm wine yeasts relatives sequence submissions to the Genbank were from China and beverages were mainly the sources of close relatives of *S. cerevisiae* and *P. kudriavzevii* whereas *C. ethanolica* closest relatives were from various non-food sources. Overall relatives of palm wine yeasts were not specific to any particular food or fermentation mix. The guanine-cytosine (G+C) content in *P. kudriavzevii* (57–58%) and *C. ethanolica* (56–57%) was higher than that of *S. cerevisiae* (47.3–51%). This suggests that the *P. kudriavzevii* and *C. ethanolica* have a higher recombination rate than *S. cerevisiae* strains analyzed. The data may help to understand palm wine yeast conservation and the diverse food matrixes and geographical origins where their close relatives exist.

Keywords: yeasts, phylogeny, *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Candida ethanolica*

1. Introduction

Palm wine is a traditional drink consumed mainly in sub-Saharan Africa, parts of Asia, and South America. It is obtained from fermentation of saps of different palm trees. Palm wine is sourced from palm trees and they grow throughout tropical and subtropical regions with just a few species found in temperate regions possibly due to freeze intolerance of seedlings [1]. The method of obtaining the drink by tapping has been described in many reports [2] and the

palm sap varies according to palm trees found in different geographical location. Yeasts are the main organisms implicated in the fermentation of the drink and they exist as natural flora on palm trees. Irrespective of the palm tree source, a common feature of the drink is that it goes sour within 24 h unless it is subjected to cold storage. The two trees from which palm wine is mostly tapped in Nigeria are *Raphia hookeri* and *Elaeis guineensis*. There is a debate on the possible origin or source of these palm trees. The tree *Raphia hookeri* is known as the wine palm and is the most widespread familiar Raphia palm in fresh water swamps of west and central Africa [3]. Many local varieties exist in the tropical rain forest of Nigeria and it is also grown in India, Malaysia, and Singapore [4]. The *E. guineensis* oil palm variety is more widely found around the world. A report pointed out that *E. guineensis* palm tree originated in the tropical rain forest region of West Africa and can be found in Cameroon, Côte d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo Angola, and the Congo [5]. It is believed in the report that during the fourteenth to seventeenth centuries, some palm fruits were taken to the Americas and from there to the Far East where it thrived. Yeast are known to reflect human history [6] hence it is possible the yeast strains found in palm wine were introduced to new regions via the plant materials introduced in those locations.

Although it is known that yeasts have been used for food and beverage fermentations [7] hundreds of years ago and domestication is believed to have been initiated before the discovery of microbes [8], the extent of genetic diversity is still under study around the world. Recent reports have shown that non-*Saccharomyces* yeasts have different oenological properties to those of *S. cerevisiae* [9]. Other reports emphasize that even though biochemical and genomic studies of *S. cerevisiae* have helped our understanding of yeasts, the other lesser known yeast species have not been fully exploited [10]. More understanding of *S. cerevisiae* and non-*S. cerevisiae* yeasts in palm wine is needed [11] in order to get more information on the capabilities of yeasts present in the drink or to probe for novel species [12]. To generate more information, molecular characterization has been used by many investigators and this has led to proper identification of new yeast strains in the drink. The diversity of yeasts from palm wine has not had much in-depth investigation and reports that show evolutionary trees which are the basic structures necessary to establish the relationships among organisms [13] are few in literature. This chapter examines evolutionary relationships of palm wine yeasts and their close relatives based on 26S rRNA sequence data and aims to shed more light on the diversity of yeasts found in the drink.

2. Methodology

2.1. Ribosomal ribonucleic acid genes partial sequence data

In a previous study [2], partial 26S rRNA gene sequences from 18 palm wine yeast isolates were deposited under accession numbers (HG452325-42). The sequences from three yeasts genera identified in that study namely *S. cerevisiae, P. kudriavzevii*, and *C. ethanolica* from *Elaeis* sp. and *Raphia* sp. palm trees were selected and used to carry out new updated searches in this report. For *Elaeis* sp., the sequence accession numbers used were HG425336, HG425328, and HG425333 whereas HG425332, HG425338, and HG425335 were used for the *Raphia* sp. palm

tree. The current versions of the selected six sequences mentioned above were used separately for an updated search in the Genbank database. The searches were optimized for highly similar sequences and the first 100 sequences from relatives of each yeast species with the highest percent identity were marked to make a shortlist of up to 600 sequences. These sequences were examined for the features listed at the time of submission after which the countries of origin and sources were noted. Sources were classified as beverage, food, or non-food sources.

2.2. Construction of phylogenetic trees

Phylogenetic trees were constructed from the shortlisted sequences by using the molecular evolutionary genetic analysis (MEGA, version 7) computer software [14]. The software allowed a seamless transfer of the sequences from Genbank. Using the multiple sequence comparison by log expectation (MUSCLE) reported by Edgar [15], multiple sequence alignments (MSA) were constructed with the software. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model [16]. The tree with the highest log likelihood was chosen. Initial trees for the heuristic search were obtained using the maximum composite likelihood approach. Trees were drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. The nucleic acid composition of the sequences was calculated automatically by switching to the nucleic acids estimation mode of the software after which the G+C content of the sequences were calculated manually from the arginine, guanine, cytosine, and thiamine percentage distribution displayed. The MAS tool MUSCLE used assumes an equality of substitution rates among sites and takes into account differences in transitional, transversional rates, and G+C-content bias [17]. For brevity, only 20 sequences from the initial 100 relatives obtained are shown in the trees with the reference sequence.

The complete list of 600 sequences analyzed showing sources and countries of origin is available in the public repository figshare [18].

3. Results and discussion

3.1. Evolutionary relationships of palm wine yeasts and their relatives

Yeasts facilitate several industrial food fermentation processes, which often consist of a desired specific strain [19]. This may be why domestication is believed to be the main driver of specific yeast prevalence in a geographical location. The understanding of the ecological basis of yeast diversity in nature remains fragmented and cross-kingdom competition has been proposed as a method to generate industrially useful yeast strains with new metabolic traits [20]. Palm wine yeasts are yet to enjoy significant diversity study hence a look at their relatives will enable more information to be generated.

In the last decade, there has been increase in submissions of palm wine yeast sequences based on 26S rRNA genes mainly due to quality checks by academic journals. The identification of new strains is accompanied by performing a search with the basic local alignment search

tool [21] followed by submission of DNA sequences to the GenBank. According to Benson et al. [22], GenBank is a comprehensive database that contains publicly available nucleotide sequences for up to 370,000 formally described species. It is common knowledge that these submissions which contain a lot of information are generated mainly through submissions from investigators around the world. Each sequence data received is curated by the GenBank annotation staff to ensure that it is free from errors after which accession numbers are assigned.

All the sequences used in this study were the first versions submitted by investigators. The maximum likelihood method was preferred for the trees constructed because it is computationally intense and all possible trees are considered. Also the method can be useful for widely divergent groups or other difficult situations [23].

3.2. *Candida ethanolica*

The yeast *C. ethanolica* is not widely reported in palm wine. It has been reported as a non-conventional yeast which may present massive resource of yeast biodiversity for industrial applications because it has been found to be adapted to some of the stress factors present in harsh environmental [24]. In that report, it was found that *C. ethanolica* tolerated up to 7% v/v ethanol. This could be useful information for new palm wine drink development especially now that there is increasing interest in non-*Saccharomyces* yeasts with peculiar features able to replace or accompany *S. cerevisiae* during specific industrial fermentations [25].

The *C. ethanolica* strain from *Raphia* sp. (**Figure 1**) and *Elaeis* sp. (**Figure 2**) palm wine showed close relationships with other *Candida* species. The relatives of *Raphia* sp. palm wine that emanated from the same node (**Figure 1**) came from diverse sources. The flanking close relatives (KY283163 and DQ466540) of *C. ethanolica* (HG425332) were isolated from composite microbial powders for aquaculture in China [26] and composite cocoa fermentation in Ghana [27]. Other close relatives included species from the genus *Pichia.* The *P. deserticola* strain (KM005182) from the same node as the reference strain was from aerobic deterioration of total mixed ration silage in China [28]. For *Elaeis* sp. (**Figure 2**) palm wine, close relatives to *C. ethanolica* (HG425336) strain were from a laboratory culture collection with unidentified source [29] and a tannin tolerant yeasts associated with naturally fermented *Miang* leaves in Thailand [30]. A close *P. deserticola* strain of unstated source in GenBank was from a large characterization study [31].

In both *Elaeis* sp. and *Raphia* sp. palm wine, several monophyletic groups were formed with other *Pichia* species namely *P. deserticola*, *P. Manshurica* and *P. galeiformis* which indicate polyphyletic relationships. The polyphyletic nature of *Pichia* has been demonstrated by Kurtzman and Robnett [29] in the analysis of gene sequences that included all known ascomycetous yeasts. Apart from possible similar conserved regions, previous nomenclature at the time of submission of the sequences may also be the reason why *Pichia* species of different genus were observed as close relatives of *C. ethanolica* from *Elaeis* sp. and *Raphia* sp. palm trees.

It has been reported that ascomycetic fungi submitted to the database previously have been assigned names based on their life stages [32, 33]. For example, it was shown that the name for the fungi *Candida krusei* is based on the anamorphic stage whereas its telemorph stage

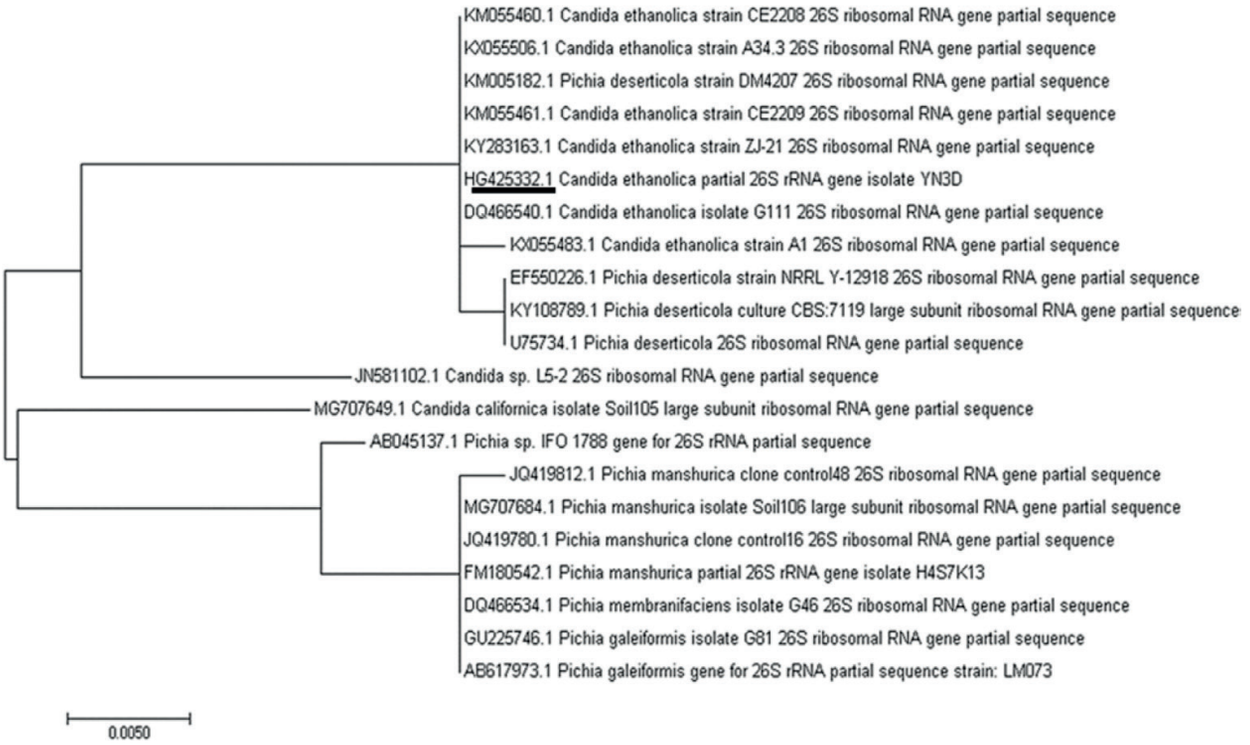

Figure 1. Phylogenetic analysis of *Candida ethanolica* (HG425332-underlined) from *Raphia* sp. palm wine. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

name is *Pichia kudriavzevii*. It also has an older name *Issatchenkia orientalis*. The whole *Candida* species consists of up to 850 organisms, which can be distantly related [34]. Hence in order to avoid the confusion, the International Botanical Congress in Melbourne in July 2011, made a change in the international code of nomenclature for fungi and adopted the principle of one fungus can only have one name and ended the system of permitting separate names to be used for anamorphs [35]. The report emphasized that this validated all legitimate names proposed for a species, regardless of what stage they were typed and can serve as the correct name for that species.

3.3. *Sachharomyces cerevisiae*

The yeast *S. cerevisiae* is generally known to be the most used microorganism in the food and drink manufacturing sector. The organism is the dominant yeast species isolated from many studies on palm wine. However, it is unclear whether *S. cerevisiae* as a species occurs naturally or exists solely as a domesticated species [36]. *S. cerevisiae* strains are genetically diverse, largely as a result of human efforts to develop strains specifically adapted to various fermentation processes. These adaptive pressures from various ecological niches may generate behavioral differences among these strains [37]. In a review [8], it was suggested that domestication in *Saccharomyces*, is most pronounced in beer strains, because they live in their industrial niche always and allow only limited genetic admixture with wild stocks and minimal contact with natural environments. Due to this restriction, it was pointed out that

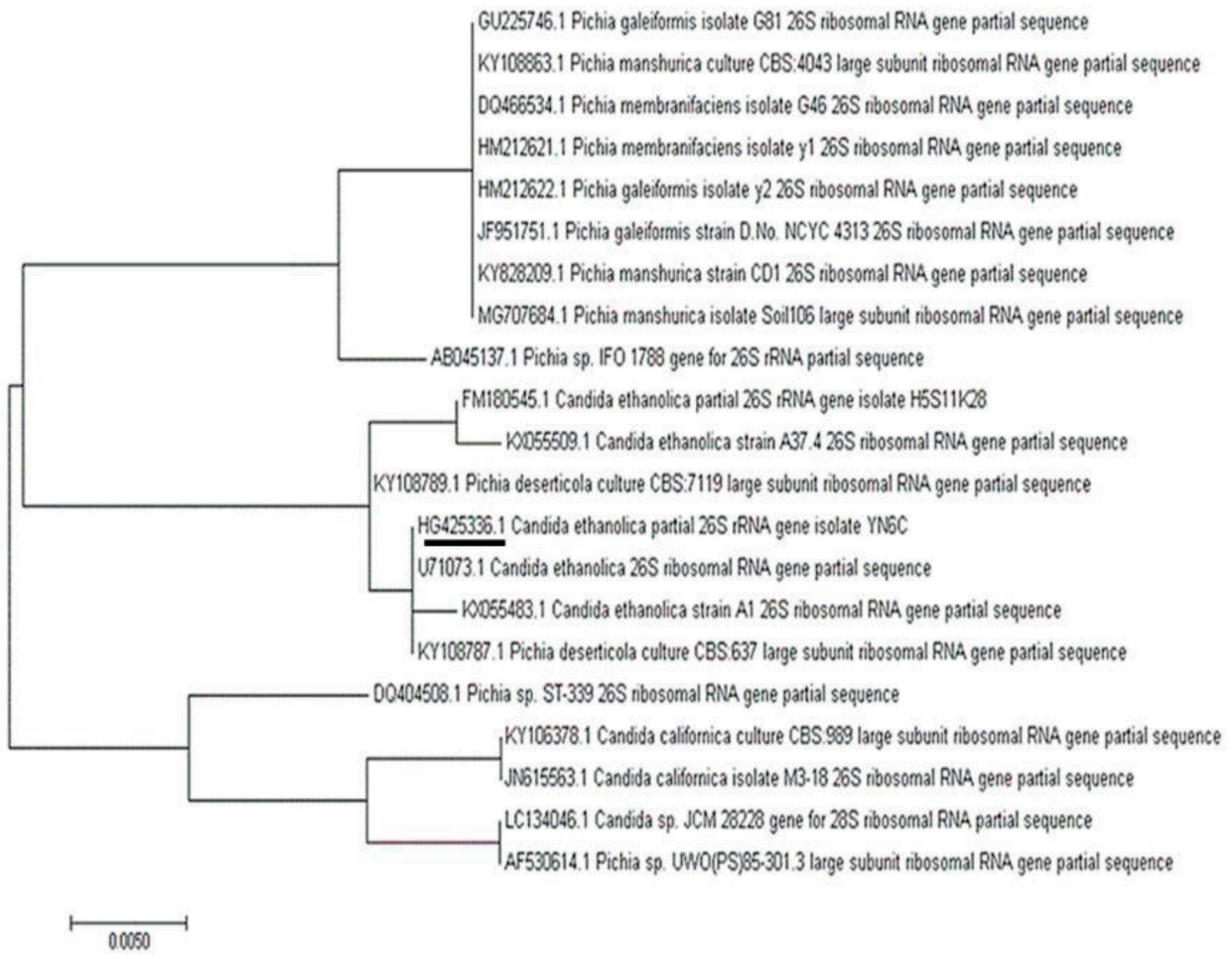

Figure 2. Phylogenetic analysis of *Candida ethanolica* (HG425336-underlined) from *Elaeis* sp. palm wine. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

beer yeast genomes show complex patterns of domestication and divergence, making both ale (*S. cerevisiae*) and lager (*S. pastorianus*) strains ideal models to study domestication.

The relatives of palm wine *S. cerevisiae* was not distributed among many species or different genus observed for *Candida species*. Two nodes were observed for the *S. cerevisiae* trees constructed for *Elaeis* sp. (**Figure 3**) and *Raphia* sp. (**Figure 4**). The yeast strain isolated from *Elaeis* sp. (**Figure 3**) was in a different branch from most of its relative whereas it was vice versa for the palm wine yeast from *Raphia* sp. (**Figure 4**) palm wine. As observed for *Candida species*, isolation of *S. cerevisiae* species was from different sources. The close relatives flanking the palm wine strain from *Elaeis* sp. palm wine (HG425328, **Figure 3**) with accession numbers KU862639 and MF966566 were isolated from grape surface [38] and pear sough dough [39] whereas the close relatives of *Raphia* sp. palm wine (HG425338, **Figure 4**) with accession numbers GU080046 and HM191669 were isolated from must of spontaneous fermentation [40] and grape juice used to brew *Musalais*, a beverage made from compressed grapes [41].

It is believed that 99% of yeasts is still unknown [42], and *S. cerevisiae* fermentation could be specific to a particular substrate, hence more studies of *S. cerevisiae* from different palm trees will be beneficial. The genus *Saccharomyces* was previously divided into two groups namely

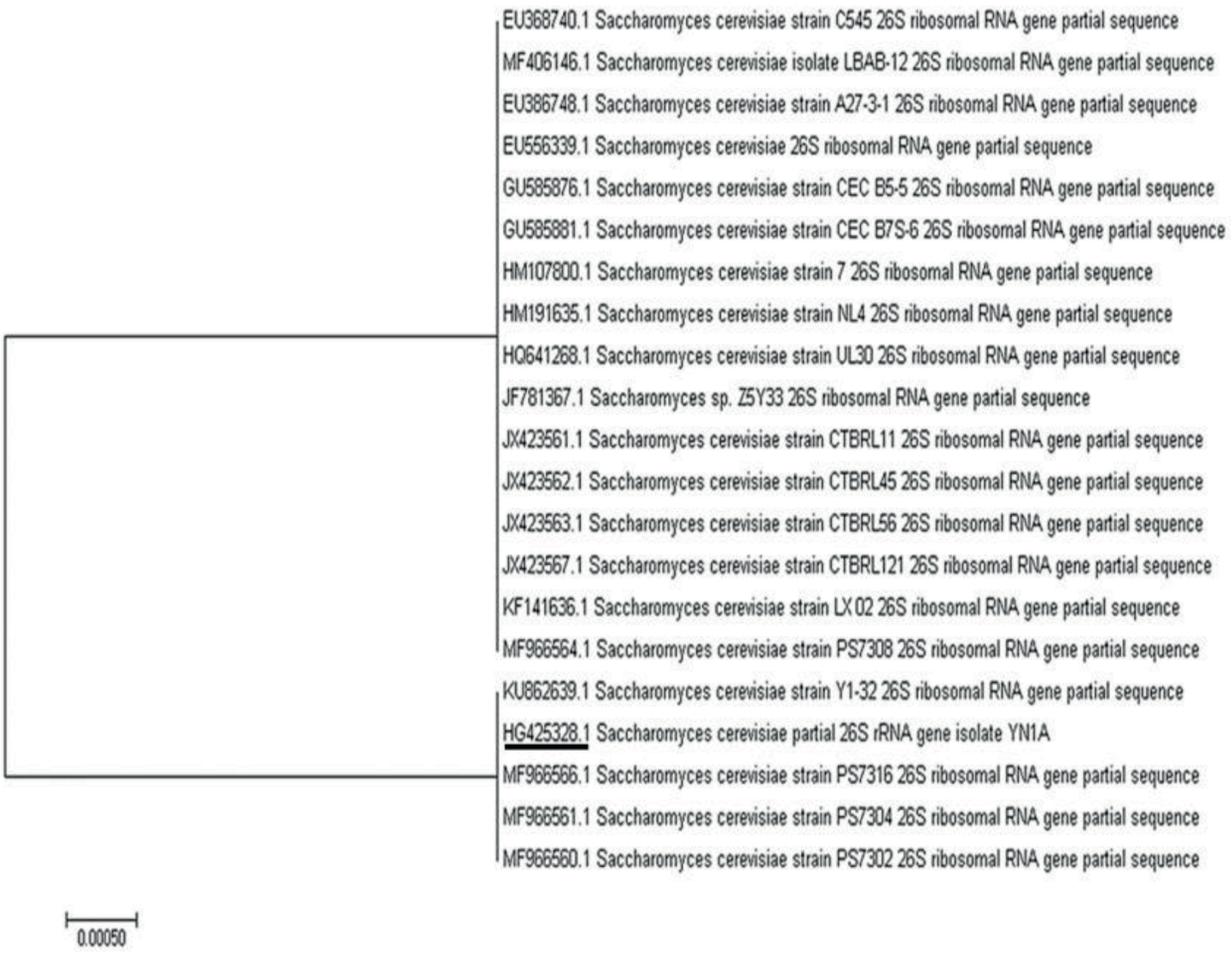

Figure 3. Phylogenetic analysis of *S. cerevisiae* (HG425328-underlined) from *Elaeis* sp. palm wine. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Saccharomyces sensu stricto and *Saccharomyces sensu lato* and the sensu stricto strains are mostly associated with the fermentation industry [43]. The *S. cerevisiae* in this study are *sensu stricto*. Comparative genomics analysis of *S. cerevisiae* and closely related species has contributed to our understanding of how new species emerge and has shed light on various mechanisms that contribute to reproductive isolation [44]. This knowledge can be applied to palm wine yeasts to ascertain how they differ from well characterized yeasts.

3.4. *Pichia kudriavzevii*

From recent molecular studies of yeasts present in palm wine, the yeast species *Pichia kudriavzevii* has emerged as a prevalent non-*Saccharomyces* yeast species in the drink. The genus has shown probiotic potentials [45] and multistress-tolerance [46]. It is worth looking closely at this genus because it has been shown that some *P. kudriavzevii* strains can produce higher quantities of ethanol from lignocellulosic biomass than conventional cells of *S. cerevisiae* at 45°C [47].

The tree constructed for *P. kudriavzevii* showed the least divergence when compared to *S. cerevisiae* or *Candida* palm wine yeast relatives. All the relatives and the *Elaeis* sp. palm wine strain (HG425333) originated from one node and formed separate taxonomic units

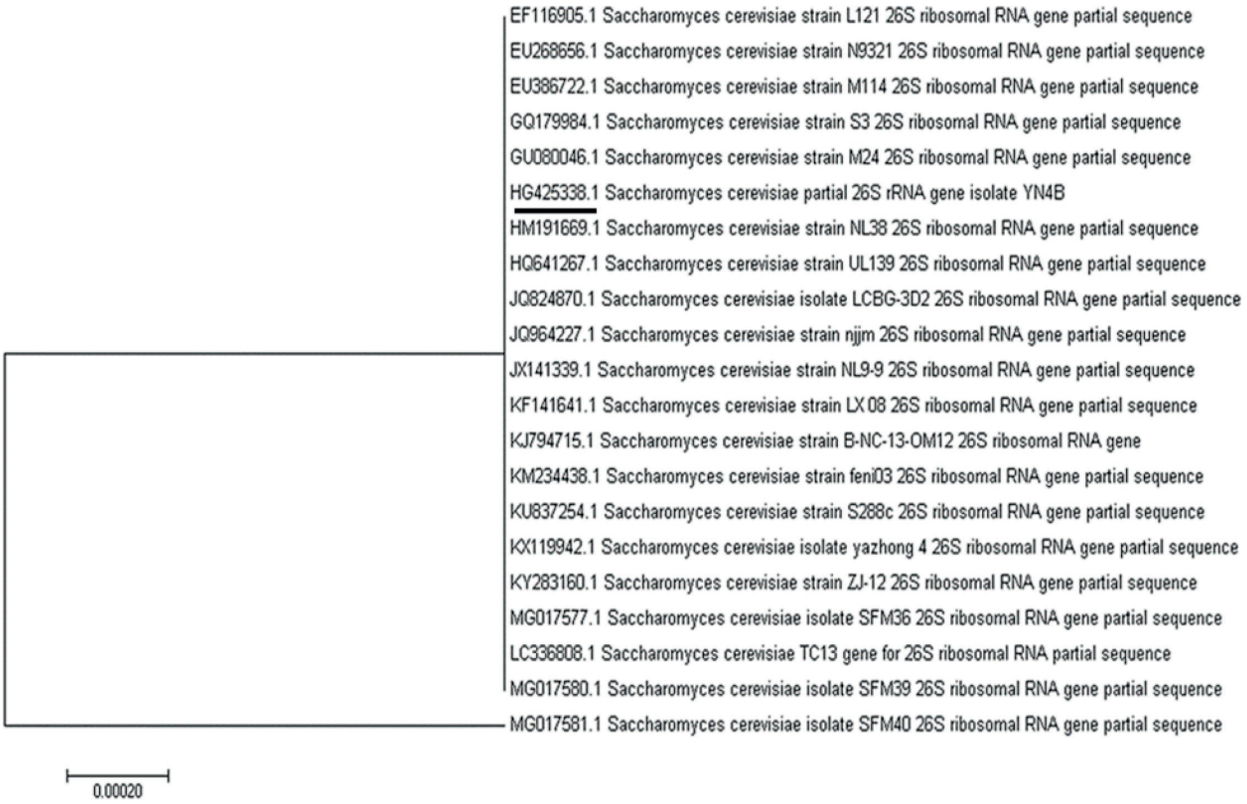

Figure 4. Phylogenetic analysis of *S. cerevisiae* (HG425338-underlined) from *Raphia* sp. palm wine. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

(**Figure 5**). In contrast, the *P. kudriavzevii* (HG425335) from *Raphia* sp. palm wine formed a separate clade and did not lie on the same branch with the relatives (**Figure 6**). This indicates intraspecies diversity and confirms findings reported previously [11]. In that study, intraspecies diversity was suggested because *P. kudriavzevii* (HG425335) from *Raphia* sp. palm wine formed a separate clade with palm wine isolates from Mexico instead of isolates from the same geographical location.

The information contained in the sequence submission of close relatives of *P. kudriavzevii* strains also shows different sources of isolation. The strains close to the yeast from *Elaeis* sp. palm wine (HG425333, **Figure 5**) with accession numbers KY283159 and KM234455 show that isolation was from composite microbial powders for aquaculture [21] and naturally fermented cashew apple juice [48] whereas a close relative of *Raphia* sp. palm wine (HG425335, **Figure 6**) with accession number KU167717 was isolated from activated sludge from textile dyeing [49].

3.5. Geographical origin and sources of palm wine yeast relatives

After ascertaining the sources of very close relatives from the phylogenetic trees constructed, the shortlisted 600 sequences from the aforementioned yeast genera were further examined and the information found was used to group the isolates according to country of isolation, food, beverage, and non-edible source.

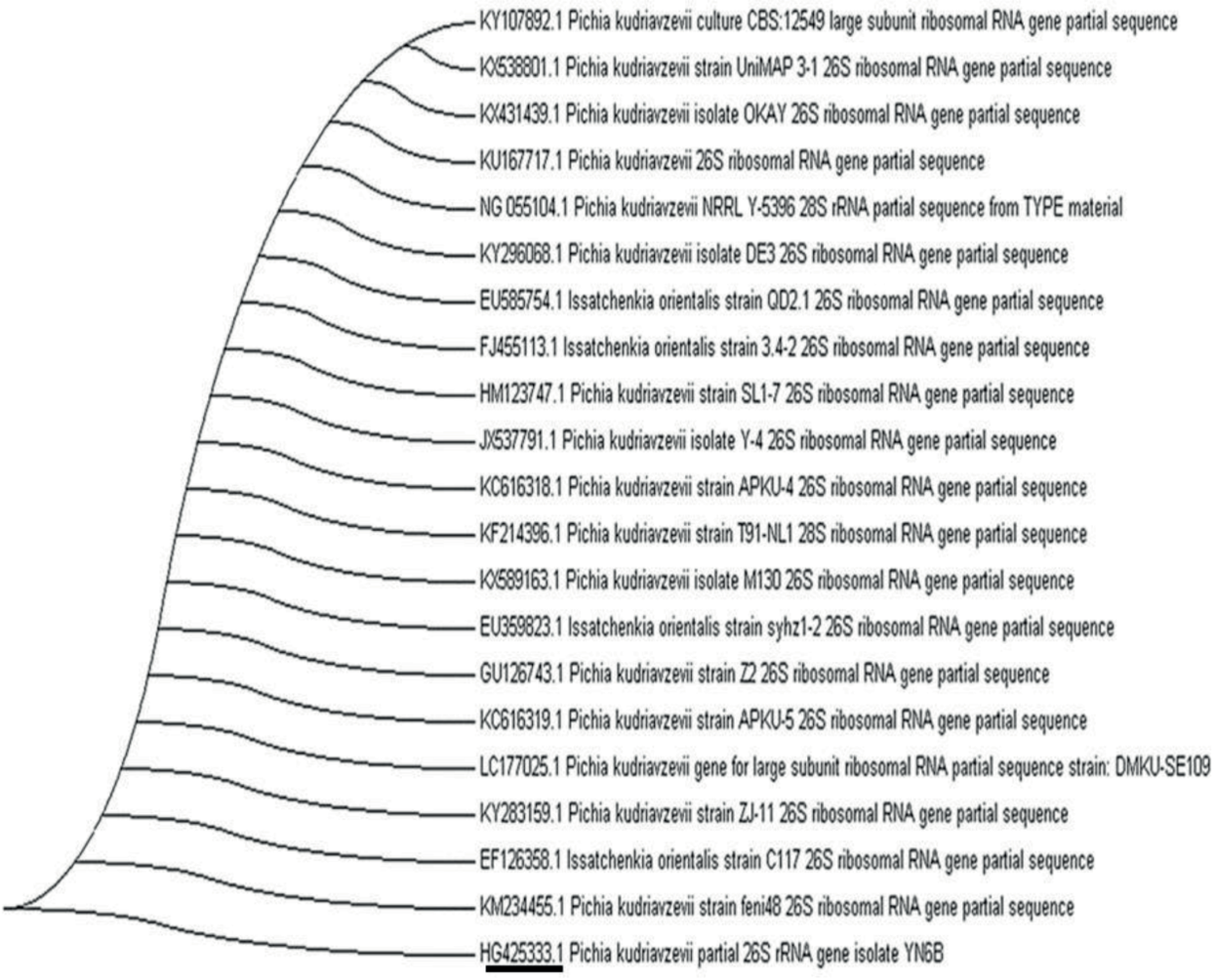

Figure 5. Phylogenetic analysis of *P. kudriavzevii* (HG425333-underlined) from *Elaeis* sp. palm wine. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

3.5.1. Isolates submitted by country of origin and source

Overall, sequences examined for the aforementioned yeasts genera were submitted from 38 countries [18] and the top 6 countries is presented in this report. Sequence data for both *Elaeis* sp. (**Figure 7**) and *Raphia* sp. (**Figure 8**) palm trees show that highest number of submissions to the Genbank database was from China. The top three countries from which palm wine yeast relatives originated were the same for both palm tree species. This suggests that a large number of palm wine yeasts may have common ancestors with yeasts found in China. The origins or sources of palm wine yeasts relatives were spread across beverages, food, and non-food sources. The prevalence of *S. cerevisiae*, *P. kudriavzevii*, and *C. ethanolica* from these sources is shown for *Elaeis* sp. palm tree (**Figure 9**) and *Raphia* sp. palm tree (**Figure 10**). In both palm wine from *Elaeis* and *Raphia* palm trees, yeasts relatives of *S. cerevisiae* and *P. kudriavzevii* species were isolated mainly from beverage sources whereas relatives representing *C. ethanolica* species were isolated from non-food sources. The sources of isolation revealed that the closest relatives of palm wine yeasts were from various sources and not specific to any particular food or fermentation mix.

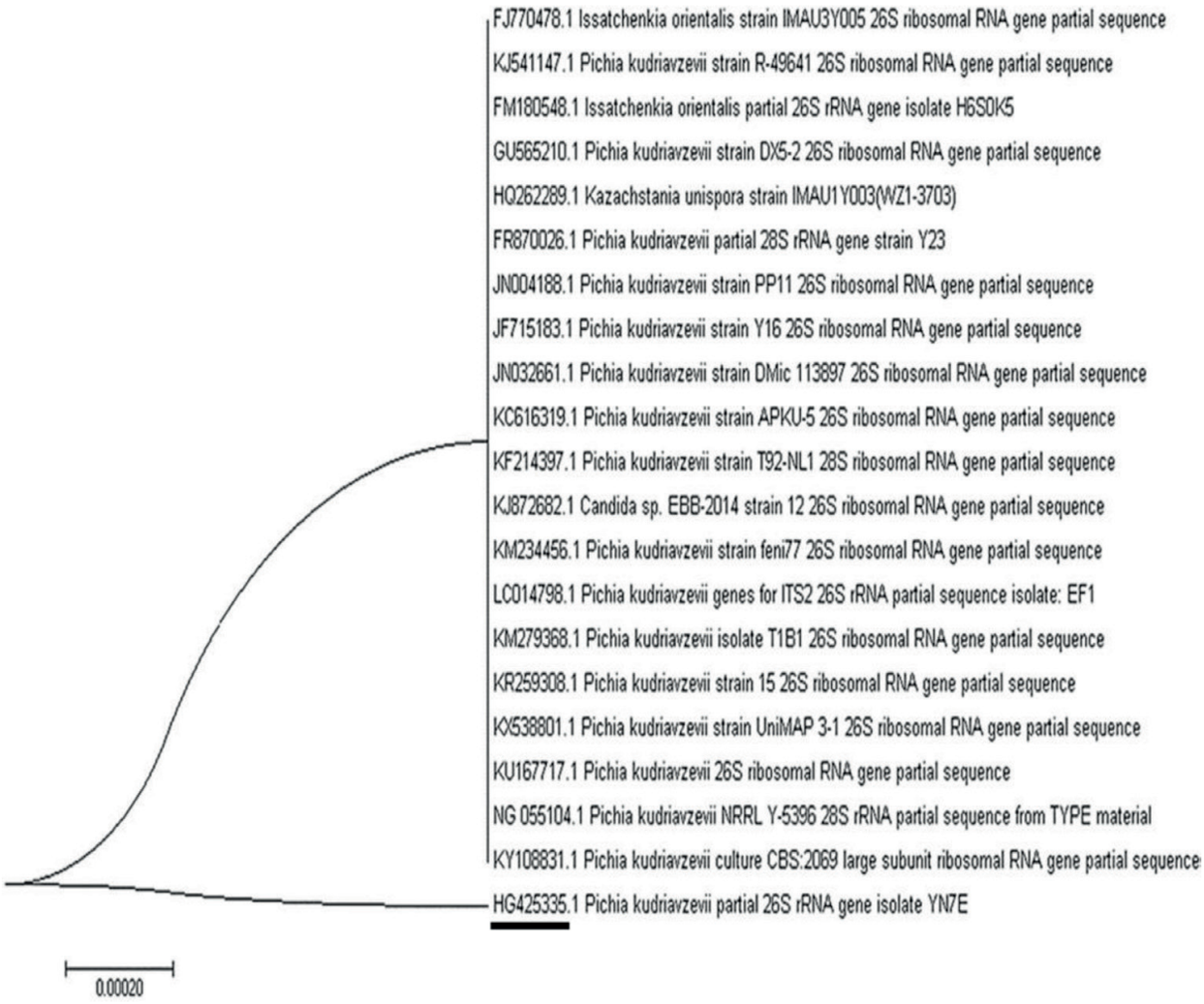

Figure 6. Phylogenetic analysis of *P. kudriavzevii* (HG425335-underlined) from *Raphia* sp. palm wine. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

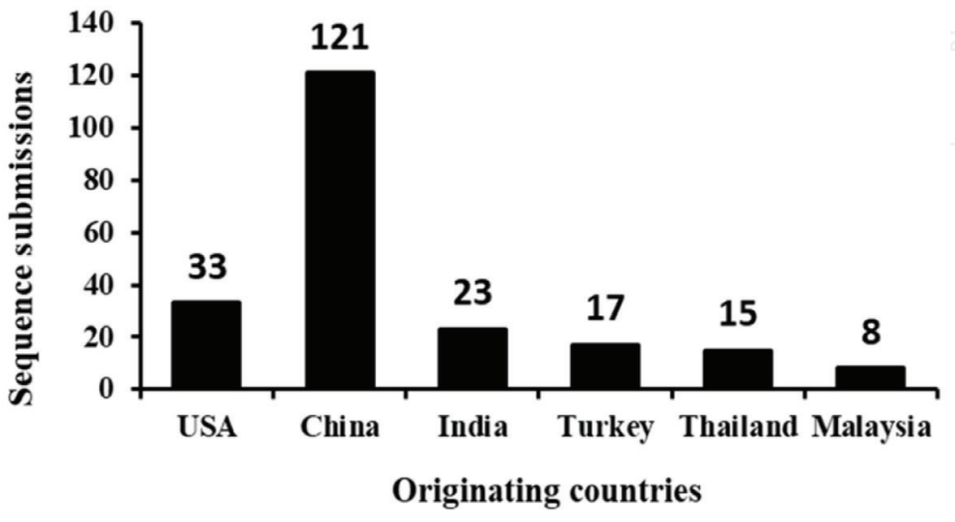

Figure 7. Top six countries from which sequences of palm wine yeast relatives of *Elaeis* sp. palm tree were submitted to the GenBank.

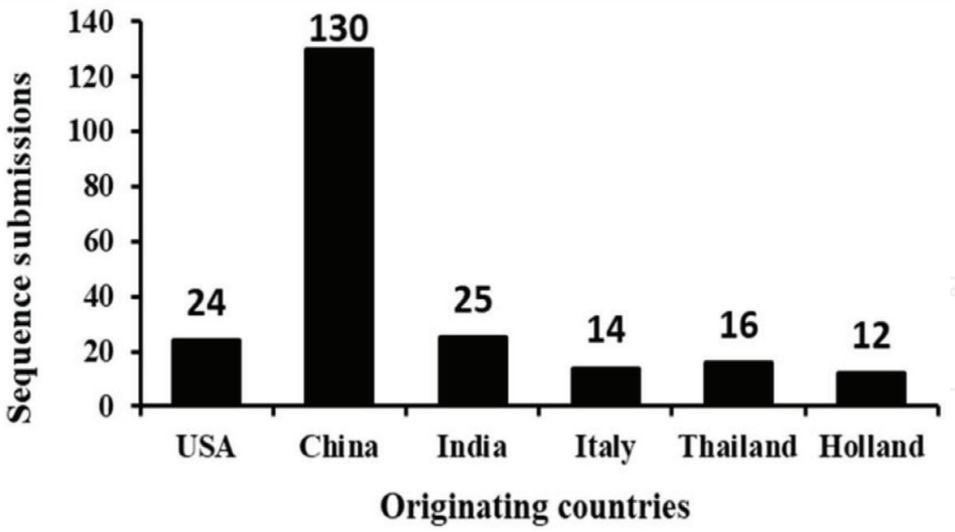

Figure 8. Top six countries from which sequences of palm wine yeast relatives of *Raphia* sp. palm tree were submitted to the GenBank.

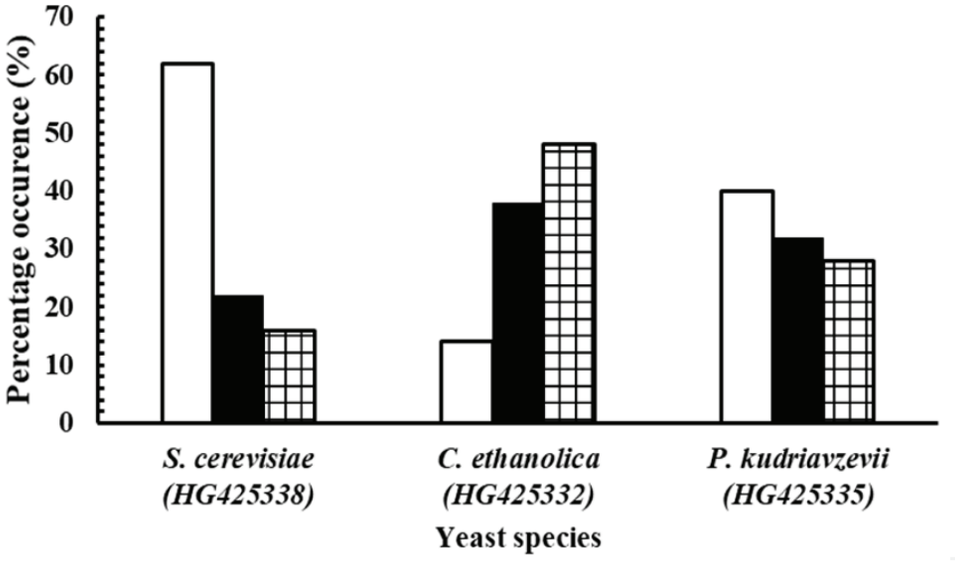

Figure 9. Distribution of palm wine yeast relatives with reference to yeasts from *Elaeis* sp. palm wine according to beverage (□), food (▮), and non-food (⊞) sources.

A report [50] found that laboratory estimates of optimum growth temperature could be used to predict global distributions of free-living microbes. Also, it was pointed out that population genetic analyses show that the genetic diversity of *S. cerevisiae* is high in the tropics and subtropics of China [51, 52]. It was suggested that without further sampling in tropical and subtropical regions, it is not possible to differentiate whether the higher diversity of *S. cerevisiae* in Asia reflects a greater habitat area or an Asian origin for *S. cerevisiae*. It would be beneficial to carry out further studies in order to establish if palm wine yeasts were taken from Africa to Asia or vice versa. The diversity could also be high in temperate regions because a study examined *S. cerevisiae* and *S. paradoxus* in northeast America and uncovered a large diversity of yeasts [53]. Up to 24 yeast isolates could not be assigned to any known species and it was suggested that the yeasts identified may be of taxonomic, medical, or biotechnological importance.

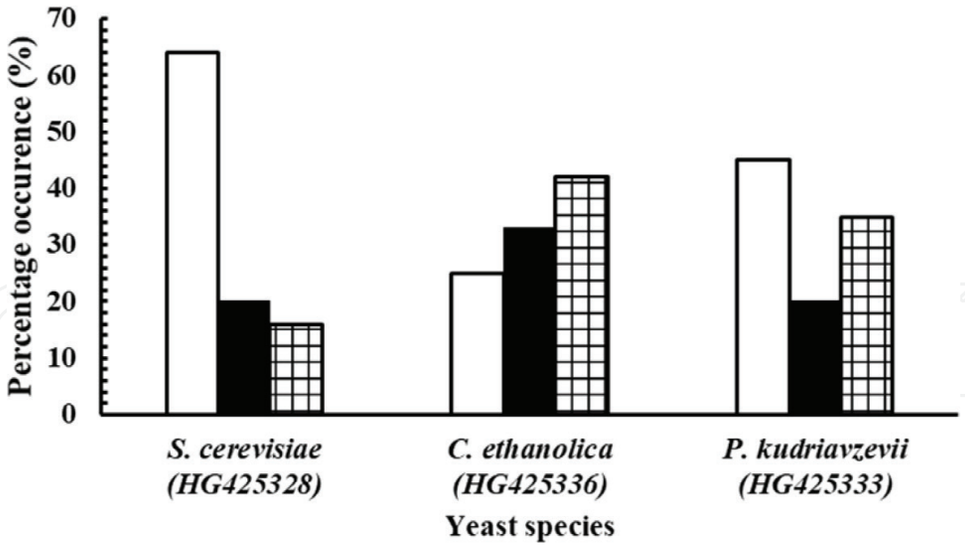

Figure 10. Distribution of palm wine yeast relatives with reference to yeasts from *Raphia* sp. palm wine according to beverage (□), food (▮), and non-food (⊞) sources.

3.6. G+C composition of palm wine yeast relatives

The G+C composition is a well known evolutionary property of eukaryotes, archaea, and bacteria. There are suggestions by Chen et al. [54], that concordance between proteomic architecture and the genetic code is related closely to genomic G+C content and phylogeny. It has been suggested that yeasts with higher G+C content have a higher recombination rate [55] and recombination is believed to be suppressed around centromeres [56]. The data in **Table 1** present the average nucleotide composition and G+C content of partial sequences of 26S rRNA genes analyzed. It shows concentration of arginine, guanine and thiamine, and cytosine concentration in *S. cerevisiae, P. kudriavzevii, or C. ethanolica* obtained from the aforementioned palm trees. Data were obtained after measuring nucleotide frequencies (%)

Yeast species	T/U	C	A	G	G+C
1. *S. cerevisiae*-R	26.3	16.6	26.5	30.7	47.3
2. *S. cerevisiae*-E	26.7	20.2	22.7	30.4	51.0
3. *P. kudriavzevii*-R	20.0	21.9	22.6	35.5	57.0
4. *P. kudriavzevii*-E	19.8	22.2	22.6	35.5	58.0
5. *C. ethanolica*-R	21.1	21.4	21.8	35.7	56.0
6. *C. ethanolica*-E	20.9	21.4	21.9	35.8	57.0

Nucleotide concentration was obtained after analysis with MEGA 7.0 software.
T/U, thiamine/uracil; C, cytosine; A, arginine; G, guanine.

Table 1. Average nucleotide composition and G+C content obtained from yeasts from *Raphia* sp. (R) or *Elaeis* sp. (E) palm wine and their relatives after measuring nucleotide frequencies (%) in 100 sequences relative to each yeast species shown.

in 100 sequences of strains relative to each palm wine yeast species listed. It was observed that the G+C content in *P. kudriavzevii* and *C. ethanolica* was higher than that of *S. cerevisiae*. This suggests that the *P. kudriavzevii* and *C. ethanolica* have a higher recombination rate than *S. cerevisiae* strains analyzed in this report. The G+C range observed is within the reported average genomic G+C-content range (13–75%) among species [57]. It was also found to be within range of G+C content (38.3–52.9%) of the *MAT* locus reported [58] in different *Saccharomycetaceae* species.

Further studies are required because G+C-content is associated with multiple biases of different nature during down stream operations and these biases may include sequencing technologies, biological, and methodological reasons [57]. Another factor that could affect the G+C content is that some yeasts like *Lachancea kluyveri* show an intriguing compositional heterogeneity in that a region of the chromosome has an average G+C content of 52.9% which is significantly higher than the 40.4% global G+C content of the rest of the genome [58].

4. Conclusions

Sequence data are useful for comparing palm wine yeasts from different trees. Data show the countries where the relatives of palm wine yeasts are dominant and may be useful for evolution and species migration studies. Palm wine yeast relatives may originate from beverage, food, and non-edible source. The G+C nucleotide data present insights on changes which may have occurred in conserved regions of some isolates over time. Comparing sequences with the highly conserved regions of the 26S rRNA genes gives an immediate picture of the lineage of palm wine yeasts and their relatives. It can also provide a foundation to select candidates for whole genome sequencing for comparision in future.

Acknowledgements

The free use of MEGA 7.0 is appreciated. Software can be accessed at https://www.megasoftware.net/home.

Author details

Ogueri Nwaiwu

Address all correspondence to: ogueri.nwaiwu@alpha-altis.co.uk

Alpha-Altis (Venture Member), Ingenuity Lab, The University of Nottingham, Nottingham, UK

References

[1] Reichgelt T, West CK, Greenwood DR. The relation between global palm distribution and climate. Scientific Report. 2018;**8**(4721):1-11

[2] Nwaiwu O, Ibekwe VI, Amadi ES, Udebuani AC, Nwanebu FC, Oguoma OI, Nnokwe JC. Evaluation of fermentation products of palm wine yeasts and role of *Sacoglottis gabonensis* supplement on products abundance. Beverages. 2016;**2**:1-13. DOI: 10.3390/beverages2020009

[3] Russell TA. The Raphia Palms of West Africa. Kew Bulletin. 1965;**19**(2):173-196

[4] Plant Use. *Raphia hookeri*—Plant resources of tropical Africa [Internet]. 2016. Available from: http://uses.plantnet-project.org/en/Raphia_hookeri_(PROTA) [Accessed: 2018-04-02]

[5] Food and Agricultural Organization. Origin of oil palm [Internet]. 2016. Available from: http://www.fao.org/DOCrEP/005/Y4355E/y4355e03.htm [Accessed: 2018-04-01]

[6] Legras JL, Merdinoglu D, Cornuet JM, Karst F. Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. Molecular Ecology. 2007;**16**:2091-2102

[7] Tamang JP, Watanabe K, Holzapfel WH. Review: Diversity of microorganisms in global fermented foods and beverages. Frontiers in Microbiology. 2016;**7**:377. DOI: 10.3389/fmicb.2016.00377/

[8] Gallone B, Mertens S, Gordon JL, Maere S, Verstrepen KJ, Jan Steensels J. Origins, evolution, domestication and diversity of *Saccharomyces* beer yeasts. Current Opinion in Biotechnology. 2018;**49**:148-155

[9] Whitener MEB, Carlin S, Jacobson D, Weighill D, Divol B, Conterno L, du Toit M, Vrhovsek U. Early fermentation volatile metabolite profile of non-Saccharomyces yeasts in red and white grape must: A targeted approach. LWT Food Science and Technology. 2015;**64**:412-422

[10] Hittinger CT, Rokas A, Bai F-Y, Boekhout T, Gonçalves P, Jeffries TW, et al. Genomics and the making of yeast biodiversity. Current Opinion in Genetics and Development. 2015;**35**:100-109

[11] Nwaiwu O, Itumoh M. Molecular phylogeny of yeasts from palm wine and enological potentials of the drink. Annual Research and Review in Biology. 2017;**20**:1-12. DOI: 10.9734/ARRB/2017/37748

[12] Nwaiwu O. Use of fragments from D1/D2 Domain of 26S rRNA gene to select *Saccharomyces cerevisiae* from palm wine. Journal of Applied Life Sciences International. 2016;**5**:1-5. DOI: 10.9734/JALSI/2016/26373

[13] Kannan L, Wheeler WC. Maximum parsimony on phylogenetic networks, algorithms. Molecular Biology. 2012;**7**:1-10. DOI: 10.1186/1748-7188-7-9

[14] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 2016;**33**:1870-1874

[15] Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. 2004;**32**:1792-1797

[16] Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution. 1993;**10**:512-526

[17] Tamura K. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Molecular Biology and Evolution. 1992;**9**:678-687

[18] Nwaiwu O. Sequence and alignment data on yeasts from palm wine and their relatives. figshare [Internet]. 2018. Available from: https://doi.org/10.6084/m9.figshare.6496676.v1 [Accessed: 2018-06-13]

[19] Steensels J, Verstrepen KJ. Taming Wild Yeast: Potential of conventional and non-conventional yeasts in industrial fermentations. Annual Review of Microbiology. 2014;**68**:61-80

[20] Zhou N, Katz M, Knecht W, Compagno C, Piškur J. Genome dynamics and evolution in yeasts: A long-term yeast-bacteria competition experiment. PLoS ONE. 2018;**13**(4):e0194911. DOI: 10.1371/journal.pone.0194911

[21] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of Molecular Biology. 1990;**215**:403-410

[22] Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Eric W, Sayers EW. Genbank. Nucleic Acids Research. 2017;**45**(Database issue):D37-D42. DOI: 10.1093/nar/gkw1070

[23] National Center for Biotechnology Information Phylogenetic Resources [Internet]. https://www.ncbi.nlm.nih.gov/Class/NAWBIS/Modules/Phylogenetics/phylo15.html [Accessed: 2018-06-20]

[24] Mukherjee M, Radecka D, Aerts G, Verstrepen KJ, Lievens B, Thevelein JM. Phenotypic landscape of non-conventional yeast species for different stress tolerance traits desirable in bioethanol fermentation. Biotechnology for Biofuels. 2017;**10**:216

[25] Steensels J, Daenen L, Malcorps P, Derdelinck G, Verachtert H, Verstrepen KJ. Brettanomyces yeasts—From spoilage organisms to valuable contributors to industrial fermentations. International Journal of Food Microbiology. 2015;**206**:24-38

[26] Zhao J. Molecular identifiation of strains isolated from composite microbial powders for aquaculture [Internet]. 2016. Available from: https://www.ncbi.nlm.nih.gov/nuccore/ky283163 [Accessed: 2018-03-01]

[27] Nielsen DS, Teniola OD, Ban-Koffi L, Owusu M, Andersson TS, Holzapfel WH. The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. International Journal of Food Microbiology. 2007;**114**:168-186

[28] Wang H. Yeasts associated with aerobic deterioration in total mixed ration silage [Internet]. 2016. Available from: https://www.ncbi.nlm.nih.gov/nuccore/km005182 [Accessed: 2018-03-03]

[29] Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek. 1998;**73**:331-371

[30] Kanpiengjai A, Khanongnuch C. Distribution of tannin tolerant yeasts associated with naturally fermented *Miang* leaves, *Camellia sinensis var. assamica* in northern Thailand [Internet]. 2016. Available from: https://www.ncbi.nlm.nih.gov/nuccore/kx055483 [Accessed: 2018-04-03]

[31] Vu D, Groenewald M, Szoke S, Cardinali G, Eberhardt U, Stielow B, et al. DNA barcoding analysis of more than 9000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. Studies in Mycology. 2016;**85**:91-105

[32] Brandt ME, Lockhart SR. Recent taxonomic developments with *Candida* and other opportunistic yeasts. Current Fungal Infection Reports. 2012;**6**:170-177

[33] Mühlhause S, Kollmar M. Molecular phylogeny of sequenced *Saccharomycetes* reveals polyphyly of the alternative yeast codon usage. Genome Biology and Evolution. 2014;**6**:3222-3237

[34] Hawksworth DL. A new dawn for the naming of fungi: Impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. Mycokeys. 2011;**1**:7-20

[35] Robert V, Vu D, Amor ABH, van de Wiele N, Brouwer C, Jabas B, et al. MycoBank gearing up for new horizons. IMA Fungus. 2013;**4**:371-379

[36] Duina AA, Miller ME, Keeney JB. Budding yeast for budding geneticists: A primer on the *Saccharomyces cerevisiae* model systemnetics. Genetics. 2014;**197**:33-48

[37] Brice C, Cubillos FA, Dequin S, Camarasa C, Martínez C. Adaptability of the *Saccharomyces cerevisiae* yeasts to wine fermentation conditions relies on their strong ability to consume nitrogen. PLoS One. 2018;**13**(2):e0192383. DOI: 10.1371/journal.pone.0192383

[38] Liu Y, Jiao J. Regional differences of grape-surface microbes significantly influence the melatonin level of wine during fermentation [Internet]. 2016. Available from: https://www.ncbi.nlm.nih.gov/nuccore/KU862639 [Accessed: 2018-05-03]

[39] Yu Y. *Saccharomyces cerevisiae* strain PS7316 26S ribosomal RNA gene, partial sequence [Internet]. 2017. Available from: https://www.ncbi.nlm.nih.gov/nuccore/mf966566 [Accessed: 2018-05-03]

[40] Zhang J. Molecular identification of wine yeasts [Internet]. 2016. Available from: https://www.ncbi.nlm.nih.gov/nuccore/gu080046 [Accessed: 2018-05-04]

[41] Zhu LX, Zhang LL, Gong MF. Analysis of 26S rDNA sequences of yeasts isolated from Musalais grape wine [Internet]. 2010. Available from: https://www.ncbi.nlm.nih.gov/nuccore/hm191669 [Accessed: 2018-05-06]

[42] Barriga EJC, Libkind D, Briones AI, Iranzo JU, Portero P, Roberts I, James S, Morais PB, Rosa CA. Yeasts biodiversity and its significance: Case studies in natural and human-related environments, ex situ preservation, applications and challenges [Internet]. 2011. Available

from: http://www.intechopen.com/books/changing-diversity-in-changing-environment/yeastsbiodiversity-and-its-significance-case-studies-in-natural-and-human-related-environments-ex-s [Accessed: 2018-05-08]

[43] Imanishi Y, Ueda-Nishimura K, Mikata K. Two newspecies of *Kazachstania* that form ascospores connected bya belt-like intersporal body: *Kazachstania zonata* and *Kazachstania gamospora*. FEMS Yeast Research. 2007;**7**:330-338

[44] Marsit S, Leducq J-B, Durand E, Marchant A, Filteau M, Landry CR. Evolutionary biology through the lens of budding yeast comparative genomics. Nature Reviews Genetics. 2017;**18**:581-598

[45] Greppi A, Saubade F, Botta C, Humblot C, Guyot JP, Cocolin L. Potential probiotic *Pichia kudriavzevii* strains and their ability to enhance folate content of traditional cereal-based African fermented food. Food Microbiology. 2017;**62**:169-177

[46] Bae J, Han J, Jeong H, Ko H, Park H, Sohn J, Sung B. Draft genome sequence of a multistress-tolerant yeast, *Pichia kudriavzevii* NG7. Genome Announcement. 2018;**6**: e01515-e01517

[47] Oberoi HS, Babbar N, Sandhu SK, Dhaliwal SS, Kaur U, Chadha BS, Bhargav VK. Ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant *Pichia kudriavzevii* HOP-1. Journal of Industrial Microbiology and Biotechnology. 2012;**39**:557-566

[48] Prabhu-Khorjuvenka SN, Doijad SP, Barbuddhe SB. Diversity of yeasts isolated from naturally fermented cashew apple juice [Internet]. 2014. Available from: https://www.ncbi.nlm.nih.gov/nuccore/km234455 [Accessed: 2018-05-07]

[49] Rosu C, Stefan A. Biodegradation of azo dyes by ascomycete yeasts [Internet]. 2017. Available from: https://www.ncbi.nlm.nih.gov/nuccore/ku167717 [Accessed: 2018-05-07]

[50] Robinson HA, Pinharanda A, Bensasson D. Summer temperature can predict the distribution of wild yeast populations. Ecology and Evolution. 2016;**6**:1236-1250

[51] Wang QM, Liu WQ, Liti G, Wang SA, Bai FY. Surprisingly diverged populations of *Saccharomyces cerevisiae* in natural environments remote from human activity. Molecular Ecology. 2012;**21**:5404-5417

[52] Almeida P, Barbosa R, Zalar P, Imanishi Y, Shimizu K, Turchetti B, et al. A population genomics insight into the Mediterranean origins of wine yeast domestication. Molecular Ecology. 2015;**24**:5412-5427

[53] Charron G, Leducq J-P, Bertin C, Dubé AK, Landry LR. Exploring the northern limit of the distribution of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* in North America. FEMS Yeast Research. 2014;**14**:281-288

[54] Chen W, Yanchun Shao Y, Fusheng CF. Evolution of complete proteomes: Guanine-cytosine pressure, phylogeny and environmental influences blend the proteomic architecture. BMC Evolutionary Biology. 2013;**13**:21. DOI: https://doi.org/10.1186/1471-2148-13-219

[55] Bradnam KR, Seoighe C, Sharp PM, Wolfe KH. G+C content variation along and among *Saccharomyces cerevisiae* chromosomes. Molecular Biology and Evolution. 1999;**16**:666-675

[56] Lynch DB, Logue ME, Butler G, Wolfe KH. Chromosomal G+C content evolution in yeasts: Systematic interspecies differences, and GC-poor troughs at centromeres. Genome Biology and Evolution. 2010;**2**:572-583

[57] Romiguier J, Roux C. Analytical biases associated with GC-content in molecular evolution. Frontiers in Genetics. 2017;**8**:16. DOI: 10.3389/fgene.2017.00016

[58] Payen C, Fischer G, Marck C, Proux C, Sherman DJ, Coppe'e J-Y, Johnston M, Dujon B, Neuveglise C. Unusual composition of a yeast chromosome arm is associated with its delayed replication. Genome Research. 2009;**19**:1710-1721