



## **Isolation and Molecular Characterization of Biosurfactant-Producing Yeasts from Saps of *Elaeis guineensis* and *Raphia africana***

**I. V. Nwaguma<sup>1\*</sup>, C. B. Chikere<sup>2</sup> and G. C. Okpokwasili<sup>2</sup>**

<sup>1</sup>World Bank African Centre of Excellence, Centre for Oil Field Chemicals Research, University of Port Harcourt, River State, Nigeria.

<sup>2</sup>Department of Microbiology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors IVN and GCO conceived the study. Author IVN carried out the laboratory analysis. Authors IVN, GCO and CBC participated in the study design, coordination and drafting of the manuscript. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aim:** This study investigated the screening and molecular characterization of biosurfactant-producing yeasts from saps of *Elaeis guineensis* (oil palm) and *Raphia Africana* (Raphia palm).

**Methodology:** Physicochemical characteristics (pH, temperature, alcohol contents, and reducing sugars) of the saps of *Elaeis guineensis* and *Raphia africana* were determined. The capacity of the yeast isolates from both samples to produce biosurfactant was evaluated using emulsification index (E<sub>24</sub>), emulsification assay, haemolytic assay, oil displacement test, and tilted glass slide. The yeast isolates were identified based on their phenotypic, microscopic, biochemical, and molecular characteristics.

**Results:** Chemical analysis of the palm wine saps revealed respective pH, temperature, alcohol, and reducing sugars contents of 5.68, 17.1°C, 0.943% and 1.090 mg/mL for *Elaeis guineensis* and 5.26, 16.9°C, 0.884% and 2.099 mg/mL for *Raphia africana*. Six isolates (SA-2, SA-5, SB-3, SB-5,

\*Corresponding author: E-mail: [ijeomanwaguma@gmail.com](mailto:ijeomanwaguma@gmail.com), [nwaguma.vivian@aceuniport.org](mailto:nwaguma.vivian@aceuniport.org) ;

SB-6 and SB-8) out of sixteen isolates (16) distributed within both samples were found to produce biosurfactant. Phylogenetic analysis based on the internally transcribed spacer (ITS) genes classified the six isolates as *Candida haemulonis* SA2, *Pichia kudriavzevii* SA5, *Pichia kudriavzevii* SB3, *Pichia kudriavzevii* SB5, *Pichia kudriavzevii* SB6, and *Pichia kudriavzevii* SB8. The sequences obtained from the study have been deposited in GenBank under the accession numbers MN007219.1-MN007224.1. The result obtained from the study revealed high biosurfactant activity with a maximum E24 of 64.5% compared to E24 of 72% by sodium dodecyl sulphate (SDS).

**Conclusion:** The study demonstrated that saps from *Elaeis guineensis* and *Raphia africana* were suitable sources of biosurfactant-producing yeasts with high capacity for hydrocarbon emulsification. The main six biosurfactant-producing yeasts were found to belong to the genera *Candida* and *Pichia*.

**Keywords:** Biosurfactant; yeasts; molecular characterization; *Elaeis guineensis*; *Raphia africana*.

## 1. INTRODUCTION

There is decreased emphasis on the use of chemically synthesized surfactants because of their adverse environmental effects: high toxicity and non-biodegradability. This has opened up interest in biosurfactant. Biosurfactants are structurally amphiphilic in nature; they tend to accumulate at interfaces between fluid phases with different polarities (e.g., oil-water or air-water), thereby reducing surface and interfacial tensions [1]. They are important because of their domestic and environmental applications. Advances in biotechnology and increased awareness on environmental protection, has encouraged their use over synthetic surfactants. They possess characteristics such as: high foaming capacity, bioavailability, biodegradability, non-toxic nature, environmental friendliness, ease of production from low cost substrates, ability to withstand high temperature, salinity and pH, and multi-functionality and specificity in terms of industrial applications [2].

Interest in the production of biosurfactant from yeasts has been on the rise. According to Amaral et al. [3], biosurfactants from yeast may offer more advantages because of yeasts' "generally regarded as safe (GRAS)" status and ability to produce higher quantities than bacteria. The latter attribute is relevant in scaling up production for industrial purpose. Organisms with GRAS status are non-pathogenic, thus allowing the application of their products in the food and pharmaceutical industries [3]. The most prevalent examples of biosurfactant-producing yeasts are *Candida* spp. [4,5,6], *Pichia anomala* [7], *Pichia fermentans* [8], *Trichosporon asahii* [9], *Rhodotorula glutinis*, *Issatchenkia orientalis*, and *Candida rugosa* [10], *Yarrowia lipolytica* [11,12] and *Pseudozyma* [13].

Furthermore, for ecologically safe biosurfactant production, the producing microbes must originate from ecologically friendly source. Most of the sources of biosurfactant-producing microorganisms do not exclude the use of pathogenic organisms. This study is unique as it reports the production of biosurfactant from yeasts isolated from completely safe sources. The use of palm wine as the source of yeasts for the production of biosurfactants is a step towards ecological acceptability of the biosurfactants produced. This was confirmed by the works of Goel [14] and Oleszek and Hammed [15].

Conventionally, yeasts have been identified based on their morphological, physiological and biochemical characteristics but these techniques seem strenuous and occasionally misleading [16]. The use of molecular and phylogenetic evolutionary approaches has immensely improved classification of yeasts as they are rapid and sensitive. Some organisms that are placed in an unqualified group are now classified appropriately owing to the emergence of molecular techniques [17]. Indeed, the non-coding internally transcribed spacer (ITS) regions are being successfully used for the identification of many yeast species [18,19], hence their use in this study. This research is unique as it investigates production of biosurfactants by yeasts isolated from palm wine (oil palm and *Raphia* palm).

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Fresh palm wine samples obtained from oil palm (*Elaeis guineensis*) and *Raphia* palm (*Raphia africana*) used for the yeasts isolation were collected from tappers early in the morning.

These samples were transported to the laboratory in sterile 500 mL sample containers under ice pack conditions and stored at 4°C until further processing. Sampling was done on two different locations: Bunu, and Kpite community within Tai Local Government Area (Ogoni land) of Rivers State, Nigeria.

## 2.2 Chemical Analysis of Palm Wine

Chemical parameters analysed were pH, temperature, specific gravity, ethanol content, total dissolved solids at 25°C, salinity at 25°C, reducing sugar and conductivity at 25°C as described by Ukwuru and Awah [20], and titrable acidity Nwachukwu et al. [21].

## 2.3 Isolation of Yeasts

For the isolation of hydrocarbon-degrading yeasts, 2% (v/v) of crude oil was added into a 250 mL conical flask, containing 100 mL of each palm wine sample as a source of carbon for enrichment. The pH of the medium was adjusted to 6. The conical flasks were then incubated at 28°C, under 150rpm shaking condition for 7 days and 14 days, respectively. A volume of 1 mL of enriched palm wine was used for serial dilution according to Nandhini and Josephine [22] and 0.1 mL aliquots from 10<sup>-3</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup> were spread-plated in triplicates on potato dextrose agar (PDA) plates containing 0.05 mg/mL of gentamycin and chloramphenicol (0.1% wt./v) to inhibit bacterial growth. The plates were incubated at 28°C for a maximum of 48h [23]. The selected colonies (confirmed to be yeasts by microscopic examination) were purified by re-streaking on PDA agar plates to obtain pure cultures. The pure isolates were maintained in PDA agar slants and sub-cultured at intervals for the various experiments conducted in this work.

## 2.4 Screening for Biosurfactant Production

The following techniques: oil spreading, emulsification index, emulsification assay, tilted glass slide, and haemolytic assay were used to evaluate potential yeast isolates for biosurfactant production as previously reported by Nwaguma et al. [24]. The selection of biosurfactant producer was based on the ability of a given strain to give positive results in all the screening methods employed.

### 2.4.1 Oil spreading test

This method is rapid and very easy to perform and most reliable in detecting diverse biosurfactant-producing microorganisms [25,26]. Isolated yeast strains were inoculated into 250 mL Erlenmeyer flask each, containing 100 mL of the culture medium with the following ingredients: KH<sub>2</sub>PO<sub>4</sub>, 0.03 g; MgSO<sub>4</sub>, 0.03 g; NaNO<sub>3</sub>, 0.3 g; yeast extract, 0.1 g, 4% of olive oil as carbon source, and the pH was adjusted to 6. The conical flasks were then incubated at 28°C under 180 rpm for 7 days. The method suggested by Morikawa et al. [27] was employed. In brief, 20 µL of crude oil was used in making a thin layer onto a petri plates [100 mm by 15 mm] containing 50 µL of distilled water. A volume of 10 µL of the cell free broth was delivered onto the oil coated surface; a clear zone on the surface indicated a positive results. The diameter of the clear zone was measured and compared with that obtained with Sodium dodecyl sulphate [SDS].

### 2.4.2 Emulsification stability (E<sub>24</sub>) test

This is one of the best and very reliable methods to measure the quantity of produced biosurfactant. The E<sub>24</sub> was determined as described by Nitschke and Pastore [28], a mixture of 2mL of kerosene and 2 mL of cell free broth obtained after centrifugation of culture were taken into a test tube and homogenized by vortexing for 2 min. After 24h, the emulsification activity was calculated using the following formula:

$$E_{24}(\%) = \frac{\text{height of the emulsified layer} \times 100}{\text{total height of the liquid layer}}$$

The emulsion formed by the cell-free broth was compared with that formed by 10% (w/v) sodium dodecyl sulphate (positive control) and distilled water (negative control), respectively.

### 2.4.3 Emulsification assay

According to Patil and Chopade [29], three milliliters (3 mL) of culture broth centrifuged at 10,000 rpm for 15 mins was mixed with 0.5 mL of hydrocarbon (kerosene). The mixture was vortexed vigorously for 2 min for homogeneity. It was left undisturbed for 1h to separate aqueous and oil phase. The spectrophotometry absorbance of the aqueous phase was measured at 600nm. Un-inoculated broth was used as blank.

#### 2.4.4 Tilted glass slide test

This is effectively a modification of drop collapse method. Persson and Molin [30] stated that a sample of colony grown for 24h on agar plates was mixed with a droplet of 0.9% NaCl at one end of the glass slide. The slide was tilted; biosurfactant producers were detected by observation of droplet collapsing down.

#### 2.4.5 Haemolytic assay

According to Shaikh et al. [31], hemolytic activity appears to be a good screening criterion for biosurfactant-producing strain because biosurfactant producing capacity was found to be associated with hemolytic activity. Fresh single colony from the each isolated culture was taken and streaked on Nutrient agar (NA) supplemented with 5% (v/v) fresh blood according to Banat [32] and Carrillo et al. [33]. The plates were incubated at 37°C for 24h. The plates were then observed for the presence of clear zone around the colonies.

### 2.5 Identification of Yeast Isolates

#### 2.5.1 Macroscopic and microscopic identification

The yeast isolates were examined macroscopically on PDA agar plates for colony elevation, pigmentation, colony size, nature and shape. For microscopy, water mount was employed; sterile distilled water was placed on a glass slide with a bacteriological loop. A light emulsion of the yeast in this drop of water was made, covered with a cover slip and examined under X40 objective lens. Biochemical features examined included urease test, carbohydrates fermentation test (glucose, galactose, sucrose, maltose, fructose, lactose, raffinose), germ tube, growth at 37°C (join together), pellicle formation and urease test.

#### 2.5.2 Molecular identification of yeast isolates

Genomic DNA of the selected pure yeast cultures was extracted with Zymo research (ZR) Fungal DNA mini prep extraction kit (Inqaba Biotech, South Africa). Freshly extracted DNA sample was vortexed on the Cetech XH-B Vortex and 2 µL dropped on the neatly wiped pedestal of the Thermo Scientific Nano drop ND-1000 Spectrophotometer for DNA quantification and

dilution of the DNA material in nanogram per litre (ng/L) before amplification via PCR. The reaction components were assembled and mixed on ice and quickly transferred to ABI 9700 Applied Biosystems thermal cycler preheated to denaturation temperature 94°C. The amplification of the 18S rRNA genes of the isolates was carried out using ITS 1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4R: 5'-TCCTCCGCTTATTGATATGC-3 primers (Inqaba, South Africa) at a final volume of 30 microliters for 35 cycles. The PCR mix included: the X2 Dream taq Master mix (Inqaba, South Africa) (taq polymerase, DNTPs, MgCl<sub>2</sub>), and the primers at a concentration of 0.5 µL, mixed with 0.5 µL of the extracted DNA template. A volume of 10.5 µL sterile nuclease-free water was added. The PCR conditions were as follows: Initial denaturation, 68°C for 30s; denaturation, 68°C for 30s; annealing, 55°C for 30s; extension, 68°C for 1 min for 35 cycles and final extension, 68°C for 5 min [34]. The product was resolved on a 1% agarose gel at 120V for 15min and visualized on a blue light trans-illuminator. The amplified products were also purified using DNA clean and concentrator (DDC) kits (Zymo research institute, South Africa), before being made ready for sequencing. The amplified 18S DNA products were sequenced on a 3500 genetic analyzer using the Bigdye Termination technique by Inqaba Biotechnology, South Africa. The sequences generated by the sequencer were visualized using ChromasLite for base calling, BioEdit was used for sequence editing [35] and Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) performed using NCBI (National Centre for Biotechnology Information) database. Similar sequences were downloaded and aligned with Clustal W and phylogenetic tree drawn with MEGA 6 software.

### 3. RESULTS AND DISCUSSION

#### 3.1 Chemical Analysis of *Elaeis guineensis* and *Raphia africana*

The chemical properties of the palm wine are presented in Table 1. The sap from oil palm had temperature of 17.1±1.27°C and pH value of 5.68±0.03, whereas that from *Raphia* palm had a temperature of 16.9±1.06°C and pH value of 5.26±0.2, at the point of collection. The pH values for both sources decreased to 3.86±0.1 and 3.56±0.6, respectively after 6h.

**Table 1. Chemical properties of the saps of palm wine**

Parameters	Palm wine saps of oil palm	Palm wine saps of Raphia palm
Temperature (°C)	17.1±1.27	16.9±1.06
pH	5.68±0.03	5.26±0.2
pH (after 6h interval)	3.86±0.1	3.56±0.6
Alcohol content (%)	14.04±0.15	13.18±0.11
Alcohol content after 6h interval (%)	15.74±0.27	15.01±0.3
Reducing sugar (mg/mL)	0.51±0.03	1.01±0.01
Reducing sugar after 6h interval (mg/mL)	0.50±0.02	0.90±0.03
Specific gravity (kgm <sup>-3</sup> )	0.827±0.024	0.903±0.05
Titrateable acidity	2.3 mL of NaOH	2 mL of NaOH
Conductivity (25°C) (µS/cm)	2.67±0.33	2.97±0.47
Total dissolved solid (TDS)@ 25°C (mg/L)	1355±28.28	1518±9.71
Salinity (25°C) (‰)	1.4±0.56	1.5±0.14

### 3.2 Selection and Identification of Biosurfactant-producers

Out of the 16 yeast isolates screened (Table 2), 6 isolates were selected as the biosurfactant producers based on their ability to give positive results to all the screening techniques employed. Table 3 presents the distribution of yeasts isolates within the different palm wine saps. Eleven (11) isolates were obtained from Raphia palm while five (5) isolates were from oil palm. Table 4 shows the 6 selected isolates and their screening characteristics. The isolate codes were

SA-2, SA-5, SB-3, SB-5, SB-6, and SB-8. Two (2) isolates from oil palm were positive to all the screening tests while four (4) isolates from Raphia palm gave positive results to all the screening tests. The cultural and colonial characteristics of the seven biosurfactant-producing isolates are shown in Table 5. Table 6 displays the biochemical characteristics of the biosurfactant-producing yeast isolates. Microscopically, using wet mount, budding yeast-like cells, ovoid and elongated in shape were seen.

**Table 2. Screening results of the selected yeast isolates**

Isolate code	Source	Emulsification index (E <sub>24</sub> )% (using kerosene)	Oil spreading (using crude oil) (mm <sup>2</sup> )	Haemolytic assay (mm)	Tilted glass slide test	Emulsification assay (OD <sub>600</sub> nm)
*SB3	RP	60±5.65	56±2.82	γ	+	2.015±0.26
SB1	RP	44±2.83	27±4.24	γ	-	2.283±0.02
SB14	RP	-	7.0±0.57	-	+	0.600±0.08
*SA5	OP	61.3±6.36	37±5.66	γ	+	2.156±0.06
*SA2	OP	62.5±7.78	55±7.07	γ	+	1.977±0.02
*SB6	RP	61.2±1.70	48±2.82	γ	+	1.854±0.14
SB11	RP	58.8±6.51	33±4.24	γ	-	2.308±0.13
*SB8	RP	65.6±6.22	74±5.66	γ	+	3.000±0.28
SB4	RP	-	-	-	-	0.240±0.01
SA7	OP	12.9±0.14	7.0±1.41	γ	-	0.244±0.03
SA3	OP	-	36±1.41	γ	-	0.256±0.04
*SB5	RP	56.3±2.04	69±4.24	γ	+	2.083±0.04
SB2	RP	-	11±2.83	-	-	0.662±0.08
SB12	RP	-	-	-	-	0.350±0.01
*SB7	RP	64.5±6.22	33±2.80	γ	+	2.403±0.06
SA8	OP	45.2±2.97	-	γ	-	2.314±0.05

Legend: OP = oil palm; γ = gamma haemolysis; + = positive test; - = negative test; \* = isolates showing positive results in all the screening methods; and OD = optical density; Values represent mean and standard deviation for duplicate experiments

**Table 3. Distribution of the yeast isolates within the Palm wine samples**

Samples sources	Number of isolates	Cfu/mL
Raphia palm	11	4.84x10 <sup>8</sup>
Oil palm	5	2.38x10 <sup>8</sup>

Legend: Cfu/mL=colony forming unit per millilitre

**Table 4. Screening results of the selected yeast isolates**

Isolate code	Sample source	Emulsification index (E <sub>24</sub> )%	Oil spreading test (mm <sup>2</sup> )	Haemolytic assay(mm)	Tilted glass slide test	Emulsification assay (A <sub>600</sub> nm)
SA2	OP	62.5±7.78	55±7.07	γ	+	1.977±0.02
SA5	OP	61.3±6.36	37±5.66	γ	+	2.156±0.06
SB3	RP	60.0±5.65	56±2.82	γ	+	2.015±0.26
SB5	RP	56.3±2.04	69±4.24	γ	+	2.083±0.04
SB6	RP	61.2±1.70	48±2.82	γ	+	1.854±0.14
SB8	RP	65.6±6.22	74±5.66	γ	+	3.000±0.28

Legend: RP = Raphia palm; OP = oil palm; γ = gamma hemolysis; + = positive test; values represent mean and standard deviation for duplicate experiments

**Table 5. Colony morphology of biosurfactant-producing yeast isolates**

Isolate code	SA2	SA5	SB3	SB5	SB6	SB8
Size	Medium	Small	Medium	Medium	Medium	Medium
Shape	Ovoid	Elongated	Elongated	Elongated	Elongated	Elongated
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Flat	Raised	Flat	Flat	Flat	Raised
Pigment	-ve	-ve	-ve	-ve	-ve	-ve
Colour	Cream	Cream	Cream	Cream	Cream	Cream
Texture	Dry	Mucoid	Dry	Mucoid	Dry	Slimy
Surface	Dull	Dull	Smooth	Smooth	Smooth	Smooth
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque

Legend - = negative

**Table 6. Biochemical identification of the biosurfactant-producing yeast isolates**

Isolate code	SA2	SA5	SB3	SB5	SB6	SB8
Carbohydrate fermentation test						
Maltose	+A	+A	+/-	+A	-/-	+A
Lactose	-/-	-/-	-/-	-/-	-/-	-/-
Sucrose	+/-	+A	+/-	+A	+A	+A
Glucose	+A	+A	-A	+A	+A	+A
Galactose	+A	+/-	+A	+/-	-/-	+/-
Fructose	+A	+A	+/-	+A	+A	+A
Raffinose	-/-	-/-	-/-	-/-	-/-	-/-
Pellicle formation	-	-	-	+	+	+
Growth @ 37°C	-	++	+++	+	+++	+++
Germ tube	-	-	-	-	-	-
Microscopy (wet mount)	Ovoid to globose	Elongated	Ovoid to elongated	Elongated	Elongated	Elongated
Urease	+	-	-	+	+	+
Probable genus	<i>Candida</i>	<i>Pichia</i>	<i>Pichia</i>	<i>Pichia</i>	<i>Pichia</i>	<i>Pichia</i>

Legend: - = negative; + = positive; A=acid production; + at 37°C = scanty growth; ++ at 37°C = moderate growth; and +++ at 37°C = heavy growth

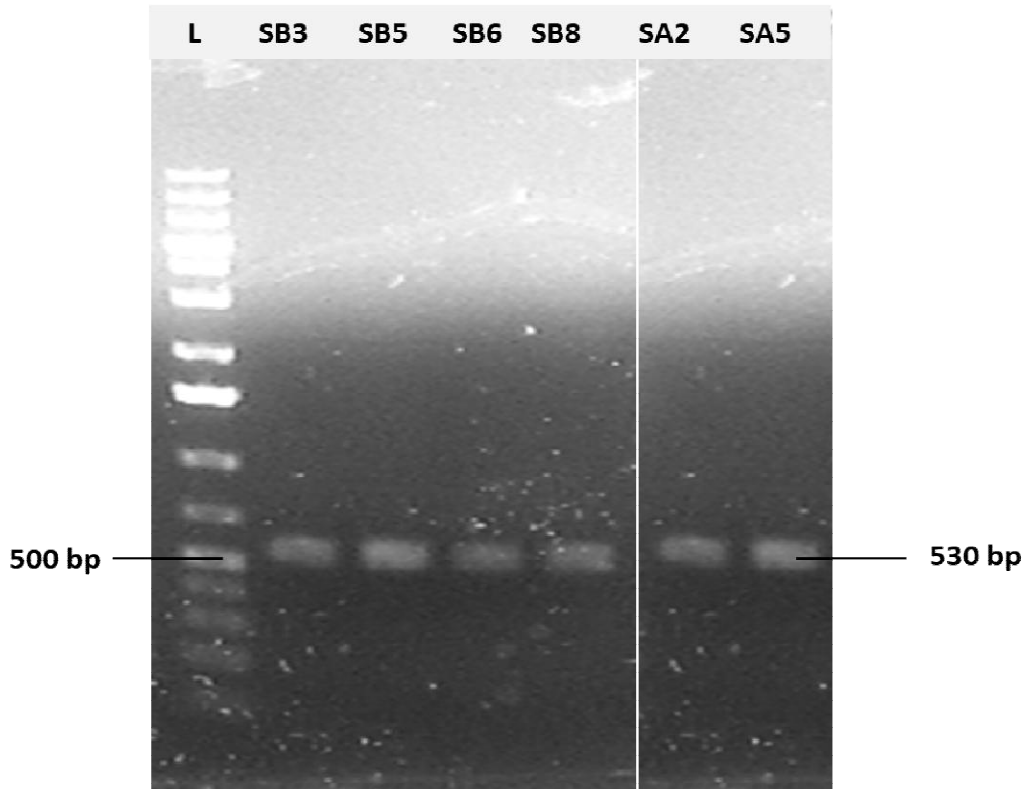
### 3.3 Molecular Characterization of the Isolates

The quantification of the genomic DNA of the six (6) isolates using the Nanodrop spectrophotometer and agarose gel electrophoresis, showed that the entire DNA extracted was pure. All the 6 isolates showed amplification with an amplicon size 530 bp. Fig. 1 shows PCR amplification images of the internally transcribed space (ITS) bands of the yeast isolates from Raphia palm and oil palm, while Fig. 2 shows the phylogenetic tree.

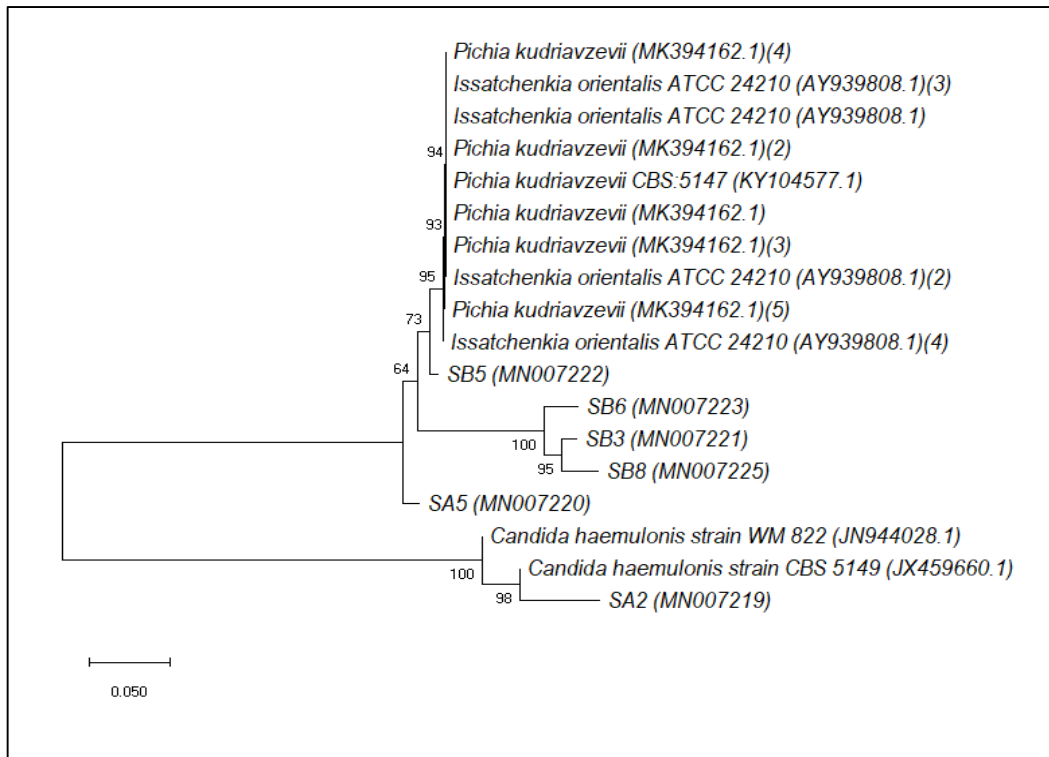
### 4. DISCUSSION

This study evaluated the isolation and molecular characterization of biosurfactant-producing yeasts from the saps of *Elaeis guineensis* (oil palm) and *Raphia africana* (Raphia palm) within Ogoniland, in Niger Delta Area of Nigeria. Many researchers have reported the isolation of biosurfactant-producing yeasts from hydrocarbon polluted sites and also from other sources. For example, Katemai et al. [36] recognized oleic

acid as a biosurfactant produced by *Issatchenkia orientalis* SR4, isolated from contaminated soil, while *Candida parasilopsis*, *Pichia anomala* and *Rhodotorula mucilaginosa* from polluted sediments produced sophorolipid [37]. Yalcin et al. [38] was able to identify nine potent biosurfactant-producing yeasts *Geotrichum candidum*, *Yarrowia lipolytica*, *Candida tropicalis*, *Galactomyces geotrichum*, *Candida tropicalis*, *Rhodotorula* sp., *Apiotrichum loubieri*, *Rhodotorula mucilaginosa*, and *Cystobasidium slooffiae* from soil samples contaminated with petroleum derivatives. Konishi et al. [39] reported that high mannosylerythritol lipids (MELs) biosurfactant-producing yeasts like *Pseudozyma antarctica* T34; *Pseudozyma tsukubaensis* JCM 10324; and *Pseudozyma graminicola* CBS10092 were randomly isolated from various vegetables and fruits. Ahmed et al. [40] reported the biosurfactant-producing ability of promising yeast isolates (*Geotrichum candidum*, *Galactomyces psuedocandidum* and *Candida tropicalis*) from fresh rhizosphere samples of healthy planted crops from different location in El-Hamoul centre in Egypt.



**Fig. 1. PCR amplification images of the internally transcribed space (ITS) bands of yeasts isolated from palm wine (oil palm and Raphia palm) (lane 1: DNA maker; lane 2-6: ITS regions of the isolates)**



**Fig. 2. Neighbour-joining phylogenetic tree of the yeast isolates**

A work done by Chandran and Das [9] showed that *Trichosporon ashii* from contaminated soil produced biosurfactant that degrades diesel oil, while *Streptomyces* sp. DPUA 1559 isolated from lichens of the Amazon has biosurfactant-producing potential according to Santos et al. [41]. The screening techniques used in this study, included both qualitative (haemolytic assay and tilted glass slide) and quantitative (emulsification index and oil spreading) methods. Satpute et al. [42] suggested that a single method is not suitable to select all the biosurfactant-producing microorganisms and recommended a combination of methods. In addition, Ndibe et al. [43] reported the confirmation of biosurfactant-production using the following classical techniques: haemolysis test, oil spreading, drop collapse, and emulsification index test. These screening methods are widely recommended for use in detecting biosurfactant-producing capability of microorganisms by other researchers. Bodour and Miller-Maier [44], Morikawa et al. [27] and Satpute et al. [42] have all reported the efficiency of oil spreading as a screening method. Cooper and Goldenberg [45] and Sarrubo [46] designed a formula for classifying biosurfactants based on emulsification activity

and emulsification assay [29,47]. Persson and Molin [30] reported the use of modified drop collapse (tilted glass slide test) to ascertain biosurfactant activity whereas, Banat [32] and Carrillo et al. [33] employed haemolytic assay for biosurfactant production.

The biosurfactant-producing yeasts were selected based on their ability to give positive results to the screening techniques employed in the study. After selection, the six (6) biosurfactant-producing yeasts were identified using biochemical and molecular approaches. The molecular identification was based on the maximum identity score in the BLAST results, homology and phylogenetic data. The SA2 yeast strain was classified as *Candida haemulonis* and had a maximum score of 96.4% identity towards *Candida haemulonis* strain CBS5149 (KU557485.1) for a query cover of 98%. The yeast strains SA5, SB3, SB5, SB6, and SB8 belonged to the *Pichia* species. Strain SA5 had maximum identity score of 96% towards *Pichia kudriavzevii* (MK394162.1) for a query cover of 98%; SB3 had a maximum score of 81.97% towards *Pichia Kudriavzevii* (MK394162.1) for a query cover of 97%; SB5 had a maximum score of 97.2% towards *Pichia Kudriavzevii*



(MK394162.1) for a query cover of 98%; SB6 had a maximum identity score with 83% towards *Pichia kudriavzevii* (MK394162.1) for a query cover of 97%; and SB8 had a maximum identity score of 80.9% towards *Pichia kudriavzevii* (MK394162.1) for a query cover of 96%. The phylogenetic trees were constructed and their relationship determined.

The oil palm had two (2) isolates (*Candida haemulonis* SA2 and *Pichia kudriavzevii* SA5), and Raphia palm had four (4) isolates (*Pichia kudriavzevii* SB3, *Pichia kudriavzevii* SB5, *Pichia kudriavzevii* SB6 and *Pichia kudriavzevii* SB8). Although, there have been reports on biosurfactant production by different *Candida* species from various sources, but there is dearth of information about biosurfactant-producing *Candida haemulonis* SA2. For example, the following *Candida* sp. have been reported for biosurfactant production: *Candida ishiwadae* [48], *Candida bombicola* [49,50], *Candida tropicalis* [38,40,51], *Candida glabrata* [52], *Candida apicola* [53], *Candida antarctica* [54], *Candida sphaerica* UCP09955 [55], *Candida utilis* [56,57], *Candida valida* [58], *Candida boleticota* [59], and *Candida lipolytica* [60].

*Pichia* spp. are very interesting yeasts but studies on biosurfactant production by *Pichia kudriavzevii* are still scarce. Some researchers have used *Pichia* strains for biosurfactant production. A study by Thanayavarn et al. [7] identified sophorolipids biosurfactant-producing *Pichia anomala* PY1 from Khao Mhak, a Thai fermented food. Johnny [61] demonstrated also, the efficacy of *Pichia fermentans* isolated from dairy effluents in producing biosurfactant. Furthermore, *Pichia anomala* CE009 from cashew nut processing plant effluent and *Pichia membranaefaciens* CE015 from an oil refinery effluent produced biosurfactant according to Martins et al. [62]. Thus, this research is unique and novel, as it is one of the first reports on biosurfactant production by *Candida haemulonis* and *Pichia kudriavzevii* obtained from saps of *Elaeisis guineensis* and *Rafia africana*.

## 5. CONCLUSION

This study demonstrated the capability of yeast isolates from saps of palm wine to produce biosurfactant. The use of palm wine as the source of the yeasts for the production of biosurfactants is a step towards ecological acceptability of the biosurfactants produced as it excludes the possibility of adverse environmental effects. Moreover, biosurfactants from yeasts

result in higher yields and yeasts have generally regarded as safe status.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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