Effect of Cultural Conditions on Biosurfactant production by *Candida* sp. isolated from the sap of *Elaeis guineensis*

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ABSTRACT

Aims: This study is aimed at determining the effect of cultural condition on biosurfactant production by *Candida* sp. isolates from saps of *Elaeis guineensis*

Methodology: Chemical analysis of the sap was carried out. Yeast isolates from the sap were screened for biosurfactant production based on emulsification index (E_{24}), emulsification assay, haemolytic assay, oil displacement test, CTAB and tilted glass slide ability. The best biosurfactant-producing yeast isolate was identified based on its phenotypic, microscopic, and biochemical characteristics. The emulsification capacity of the produced biosurfactant on selected oils was studied. Optimum cultural and nutritional requirements (temperature, pH, inoculum concentration, nitrogen sources and carbon sources) for biosurfactant production by the isolate were determined.

Results: The characteristics of the sap from *Elaeis guineensis* were reducing sugar (0.51 ± 0.03 mg/ml), alcohol (14.04 ± 0.15 %), specific gravity (0.827±0.024), and pH (5.68±0.03). The crude biosurfactant produced displaced a thin film of crude oil on petri dish by 55 mm, and revealed high emulsification index (E_{24}) of 52.5% using Olive oil as substrate compared to E_{24} of 60.6% by sodium dodecyl sulphate (SDS). Based on colonial, microscopic, and biochemical characteristics, the isolate SA2 was identified as *Candida* sp. The crude biosurfactant showed varying capacity in emulsifying the different oils that were examined. Optimization data revealed maximum biosurfactant production after 7 days of incubation, inoculum concentration of 10%, at temperature of 20 ^{o} C, pH of 2 with cassava peel as substrate.

Conclusion: The study has demonstrated the capacity of *Candida* sp. from the sap of *Elaeis guineensis* to produce biosurfactant utilizing cassava peel as substrate. The use of cassava peel, which represents a low-cost substrate, is important in reducing the cost of biosurfactant production. Moreover, using yeasts from *Elaeis guineensis* make the production process ecologically friendly.

Keywords: Biosurfactant, *Candida* sp., optimization, *Elaeis guineensis*

1. INTRODUCTION

Biosurfactants are green extracellular molecules synthesized by microorganism such as bacteria, yeasts, and fungi. They are amphipathic in nature comprising hydrophilic and hydrophobic moieties that form partitions between oil/water or air/water interfaces [1]. According to Satpute *et al*. [2], this inherent amphipathic property, increases the solubility of hydrophilic molecules, hence reducing both surface and interfacial tensions at air/water interface.

Recent preference for biosurfactants over chemically synthesized surfactant is due to its higher biodegradability, environmental friendliness, ability to withstand extreme high temperature, salinity and pH, ease of production from renewable agro-wastes, active and non-toxic nature, multi-functionality, and specificity in terms of its industrial applications [3]. These surface active molecules are classified as glycolipids (rhamnolipids, sophorolipids and trehalolipids) [4]; fatty acids and phospholipids [5]; polymeric biosurfactants (emulsan, alasan) [6-7], and particulate biosurfactants [1], based on their chemical structure and microbial origin.

Microorganisms that produce biosurfactants are naturally abundant; they are found in ecological places like land (polluted soil, sediment, sludge), water bodies (fresh water, ground water, marine water), and also in some extreme environments (e.g., oil reservoirs), where they can flourish in wide range of temperatures, pH values, and salinity [8]. In the past decades, yeast has proven their proficiency for production of biosurfactant, despite having been isolated from different sources as recorded by some researchers. This is majorly attributed to their importance in food and pharmaceutical industries on the basis of "generally regarded as safe" (GRAS) status and, also ability to produce biosurfactant in a larger quantities than bacteria [1]. The following *Candida* species: *Candida tropicalis* [9]; *Candida albicans* [10]; *Candida antarctica* [11]; *Candida bombicola* [12]; *Candida* sp. SY16 [13]; *Candida sphaerica* UCP0995 [14]; *Candida utilis* [15]; *Candida glabrata* [16], *Candida guilliermondii* [17] are known biosurfactant producers.

The type, quality, and quantity of biosurfactant depend on production process conditions such as pH, temperature, agitation, aeration, inoculum concentration, nature of substrates, carbon sources and nitrogen sources [18]. Since environmental factors may significantly affect the yield and characteristics of the produced biosurfactant, it is therefore essential to optimize the process conditions in order to achieve high yield.

Biosurfactants find application in different areas. In the environment, play vital roles in bioremediation of polluted soils and refinery wastewater and microbial enhanced oil recovery; industrially, they have been used in detergent formulation, household cleaning agent, pesticides and textile production, agriculture, food and pharmaceutical industries [19-20]. Several biosurfactants exhibits antibacterial, antifungal, antiviral and antitumor (inhibiting tumor growth and its toxic effects) properties, making them potential alternatives to conventional therapeutic agents in many biomedical applications [21-22].This work was aimed at isolating, screening and optimizing biosurfactant production from *Candida haemulonis* SA2 obtained from the sap of *Elaeis guineensis*. Finally, the ability of the biosurfactant produced to emulsify different hydrocarbons was evaluated.

2. MATERIAL AND METHODS

2.1 Sample Collection

The *Elaeis guineensis* (oil palm) samples used for the yeast isolation were obtained in a sterile 500 mL sample containers, each from a palm wine taper within 30 to 60 min of tapping. The samples were aseptically transported to the laboratory in ice packs within two hours of collection. Sampling was done on two different locations: Bunu, and Kpite community within Tai Local Government Area (Ogoni land) of Rivers State, Nigeria.

2.2 Physicochemical Analysis of Oil Palm Samples

The physicochemical parameters analysed were pH, temperature, specific gravity, ethanol content, total dissolved solids at 25 ^{o}C, salinity at 25 ^{o}C, reducing sugar and conductivity at 25 ^{o}C as described by Ukwuru and Awah [23], and titrable acidity Nwachukwu *et al*. [24].

2.3 Isolation of Yeasts

For the isolation of hydrocarbon degrading, 2 % (v/v) of crude oil was added to 100 mL of palm wine in a 250 mL conical flask. The pH of the medium was adjusted to 6. The conical flasks were then incubated at 28 ^{o}C in a shaker incubator with agitation speed of 150 rpm for 7 days and 14 days, respectively. At each of the days, 1 mL of enriched palm wine was used for serial dilution according to Nanhini and Josephine [25]; this was followed by spreading of 0.1 mL from 10^{-3}, 10^{-5} and 10^{-6} dilutions on triplicates 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 ^{o}C for 48 h [26]. The selected colonies (confirmed to be yeasts using a microscopic examination) were purified by re-streaking on PDA agar plates. The pure isolates were maintained in PDA agar slants. The isolates were sub-cultured from the slants for the various experiments conducted in this work.

2.3 Identification of Yeast Isolates

The yeast isolates were examined macroscopically on PDA agar plates for the following features, colony elevation, pigmentation, colony size, nature and shape. For microscopy, water mount was employed; with a bacteriologic loop, sterile distilled water was placed on a glass slide and a light emulsion of the yeast made in this drop of water. The glass slide was covered with a cover slip and examines under 40X objective lens. The reason is because yeast settles on a slide more quickly in an aqueous medium making it easier to measure them. The biochemical features examined were urease test, carbohydrates fermentation test (glucose, galactose, sucrose, maltose, fructose, lactose, raffinose), Germ tube test, growth at 37 ^{o}C, and pellicle formation.

2.4 Screening for Biosurfactant Production

The yeast isolates were screened for biosurfactant production using the following techniques: emulsification stability (E_{24}) test, emulsification assay, oil displacement, tilted glass slide and haemolytic assay as described by Nwaguma *et al*. [18]. The selection of the biosurfactant producer was based on the ability of a given strain to give positive results in all the screening test procedures.

2.4.1 Emulsification stability (E-24) test

This screening method for biosurfactant-producing microorganisms has been described as one of the commonest [27]. The method described by Plaza *et al*. [28], was adopted. In brief, 2 mL of kerosene was added to 3 mL of cell free broth in a test tube and vortexed at maximum speed for 2 min to homogenize the mixture. After 24h, the emulsification stability was calculated using the formula below:

$$\text{E-24} = \frac{\text{total height of the emulsified layer}}{\text{total height of the mixture}} \text{ X } 100$$

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.

2.4.2 Emulsification assay

Three millimetres of supernatant centrifuged at10000 rpm for 15 min/RT was mixed with 0.5 mL of kerosene. The mixture was vigorously homogenized by vortexing for 2 min, and was left undisturbed for 1 h to separate the aqueous and the hydrocarbon phases. The spectrophotometry absorbance of the aqueous phase was measured at 600 nm [29]. Un-inoculated broth was used as blank.

2.4.3 Oil spreading test

This method is rapid and easy to perform, and most reliable in detecting diverse biosurfactant–producing microorganisms [28, 30]. The method suggested by Morikawa *et al*. [31] was used. 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 mL of distilled water. 10 µL of cell free broth was delivered onto the oil coated surface; a clear zone on the surface indicated a positive result. The diameter of the clear zone was measured and compared with that obtained with SDS.

2.4.4 Tilted glass slide test

This is an effective modified drop collapse method [2]. A sample colony grown on nutrient agar plates for 24 h was mixed with a drop of 0.85 % NaCl at the edge of the glass slide. According to Satpute *et al*. [2], collapsing down of droplet when tilted indicated biosurfactant production.

2.5 Optimization of Cultural Conditions for Biosurfactant Production

The effects of different cultural conditions (inoculum concentration, pH, temperature, nitrogen sources and agro-wastes as carbon sources) on the growth of selected yeast isolates and the ability of the strain to produce biosurfactant were determined. The inoculum for the optimization used was standardized using 0.5 McFarland's standard.

The optimum incubation time for growth and biosurfactant production by the selected strain was studied by varying the incubation time (24, 48, 72, 96, 120, 144, and 168 h) of the culture medium. The culture medium was inoculated with a 24 h culture broth containing a total viable cell count of 2.38×10^8 cfu/ mL of the selected isolates and incubated at 28 ^{o}C for 168 h in a rotary shaker incubator. Biosurfactant production was measured using E-24 while growth was determined using a spectrophotometer. The yeast isolates were incubated at different temperature (20, 30, 40, 50 and 60 ^{o} C) for 168 h, after which the biosurfactant production and growth of the strain were determined. The inoculum concentration with different percentage such as 2, 4, 6, 8, and 10 % (v/v) was added into the culture broth, incubated for 168 h, after which the growth of yeast isolates and the production of biosurfactant were determined. The optimum pH of 2, 4, 6, 8, and 10 for the growth of the yeast isolates and biosurfactant produced were determined after incubation for 168 h. The yeast isolates were incubated using different agro-wastes (cassava peel, soya bran, sugarcane bagasse, coconut pulp and beans bran) as carbon sources, and their growth and biosurfactant production estimated after 168h of incubation. Finally, the yeast isolates was incubated with different nitrogen sources (urea + yeast extract, yeast extract + $NaNo_3$, NH_4SO_4 + yeast extract, NH_4NO_3 + yeast extract, and peptone + yeast extract for 168 h), and the growth of yeast isolates and biosurfactant production determined thereafter.

2.6 Biosurfactant Production

The optimized parameters were used in setting up the biosurfactant production media. The production was carried out in a 500 mL Erlenmeyer flask containing 300 mL of the production media with the following ingredients: KH_2P0_4, 0.03g; $MgSO_4$, 0.03g; $NaNO_3$, 0.3g; yeast extract, 0.1g, 4% of olive oil as carbon source. The conical flasks were then incubated at 28 ^{o} C under 180 rpm for 7 days

2.7 Application of the Biosurfactant on Hydrocarbon Emulsification

The biosurfactant produced was applied on different oils (soya oil, red oil, olive oil, coconut oil, orange oil, and castor oil) and the ability to emulsify these oils determined using E-24 Index.

2.8 Statistical Analysis

The results were compared by one-way analysis of variance (one-way ANOVA) and multiple range tests to find the differences between the measurement means at 5 % (0.05) significance level using IBM® and SPSS® Statistics Version 20.0 (Gally and Alder, US) [32].

3. RESULTS AND DISCUSSION

3.1 Sample Source

According to Olowonibi [1]. (2017), palm wine are naturally synthesized milky alcoholic juice from the saps of *Elaeis guineensis* (oil palm), proven to be highly nutritious, which support the growth of yeast species. Figure 1, shows the picture of milky coloured palm wine sap from oil palm.

I.

Figure 1: Sap of *Elaeis guineensis*

3.2 Physicochemical Analysis of Palm Wine Sap

The physicochemical characteristics of the palm wine are presented in Table 1. The palm wine sap had a temperature of 17.1 ^{o} C ± 1.27 and a pH value of 5.68 ± 0.03 at the point of collection. The pH value decreased to 3.8.6 after 6 h interval. The specific gravity, conductivity, salinity and total dissolved solids values @ 25 ^{o} C were 0.827 kgm^{-3}, 2.67, 1.4 % and 1355, respectively.

Table1. Physicochemical properties of the sap of *Elaeis guineensis*

Parameters	Palm wine Saps of Oil palm

Temperature (^{o}C)	17.1 ± 1.27
pH	5.68 ± 0.03
pH (after 6 h interval)	3.86 ± 0.1
Alcohol content (%)	14.04 ± 0.15
Alcohol content (after 6 h interval, %)	15.74 ± 0.27
Reducing sugar (mg/ml)	0.51 ± 0.03
Reducing sugar after 6h interval (mg/ml)	0.50 ± 0.02
Specific gravity (kgm^{-3})	0.827 ± 0.024
Titratable acidity	2.3 mL of NaOH
Conductivity (at 25 ^{o}C) (µS/cm)	2.67 ± 0.33
Total dissolved solid (TDS) @ 25 ^{o}C (mg/L)	1355 ± 28.28
Salinity (at 25 ^{o}C)(‰)	1.4 ± 0.56

3.3. Selection and Identification of Biosurfactant-producing Yeast Isolates

Out of the five (5) yeast isolates screened, two (2) isolates were selected as biosurfactant producers based on their ability to give positive results to all the screening methods employed. From the two biosurfactant-producing yeasts, the best isolate SA2 was chosen (Table 2). The distribution of yeast isolates within the different palm wine saps of *Elaeis guineensis* are shown in (Table 3). The cultural and colonial characteristics of the best biosurfactant-producing yeast isolate are shown in Figure 2 and Table 4. Table 5 presents the biochemical characteristics of the biosurfactant-producing yeast isolate. Microscopically, using wet mount, budding yeast-like cells which are ovoid in shape were seen.

Table 2. Screening results of the selected yeast isolates

Isolate code	source	Emulsification index (E_{24})% (using kerosene)	Oil spreading (using crude oil)(mm^2)	Haemolytic assay (mm)	Tilted glass slide test	Emulsification assay (OD_{600} nm)
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 ± 0.023
SA7	OP	12.9 ± 2.69	7 ± 2.83	γ	-	0.244 ± 0.010
SA3	OP	-	36 ± 8.46	γ	-	0.256 ± 0.024
SA8	OP	45.2 ± 5.94	-	γ	-	2.314 ± 0.154

*Legend: OP = oil palm; γ = gamma haemolysis; + = positive test; - = negative test; *=isolate showing positive results in all the screening methods; and OD =optical density*

3.4 Count of the Yeast Isolates within the Sap of *Elaeis guineensis*

The result obtained from the sap of *Elaeis guineensis* revealed count of 2.38x10^{8}.

Table 3. Colony morphology of biosurfactant-producing *yeast isolate*

Isolate code	*SA2*
Size	medium
Shape	Ovoid
Margin	entire
Elevation	Flat
pigment	- ve
Colour	cream
Texture	Dry

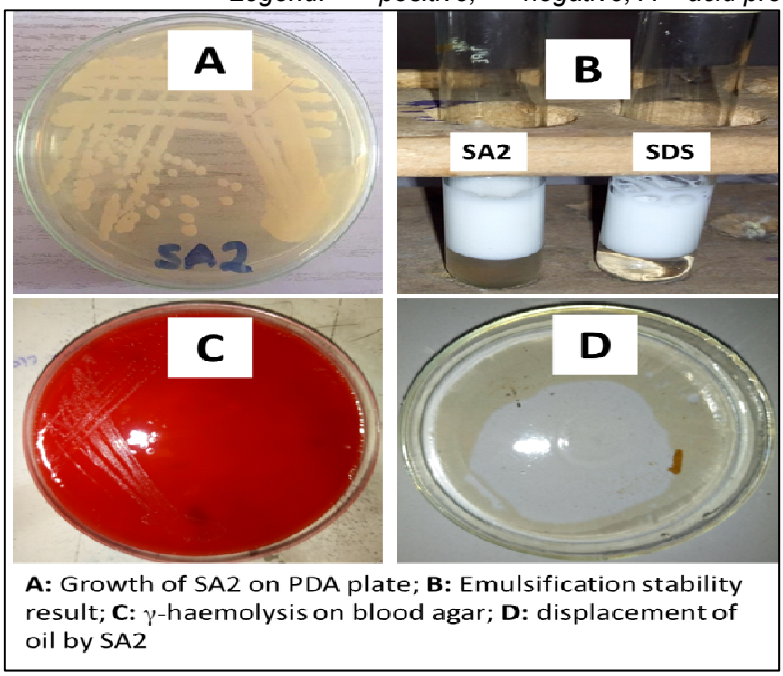
Surface	Flat & smooth
Opacity	Opaque

Legend - = negative

Table 4: Biochemical identification of the biosurfactant-producing yeast isolate

Isolate code	**SA2**
Carbohydrate fermentation	
Maltose	+/A
Lactose	-/-
Sucrose	+/-
Glucose	+/A
Galactose	+/A
Fructose	+/A
Raffinose	-/-
Pellicle formation	-
Growth @ 37 ^{o} C	-
Germ tube	-
Microscopy (wet mount)	Ovoid to globose, budding yeast-like cells
Urease test	+
Probable genus	*Candida*

Legend: + = positive; - = negative; A = acid production



A: Growth of SA2 on PDA plate; **B:** Emulsification stability result; **C:** γ-haemolysis on blood agar; **D:** displacement of oil by SA2

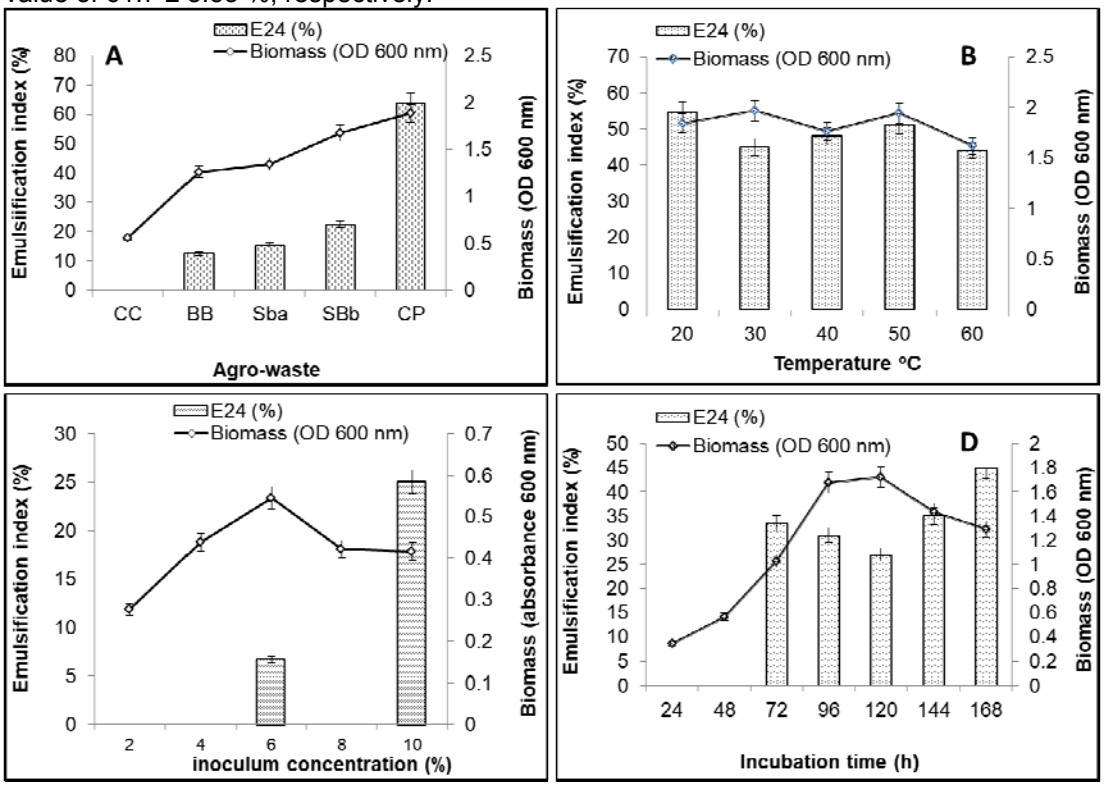
Figure 2. Growth and screening characteristics of the biosurfactant-producing isolate.

3.5 Optimization of Cultural Conditions for Improved Biosurfactant Production

Based on the analysed results, the optimum incubation time for growth and biosurfactant production were 120 and 168 h with the OD (optical density) reading of 1.720 ± 0.009 and E-24 value of 45 ± 7.07 %, respectively. Fig 1A shows the effect of different agro-wastes as

carbon sources on growth and biosurfactant production by the yeast isolate; OD reading of 0.703 ± 0.01 and E-24 value of 55.9 ± 2.82 % were obtained. Cassava peel was the best carbon source for biomass formation and biosurfactant production, with E_{24} value of 64 ± 1.41 % and OD reading of 1.8840 ± 0.01, respectively. The effect of different incubation temperatures on growth and biosurfactant production by the yeast isolate showed the optimum incubation temperatures to be 30 ^{o} C and 20 ^{o} C for growth and biosurfactant production, respectively (Fig 3B).

The effect of different percentage inoculum concentrations on growth and production of biosurfactant showed optimum inoculum concentrations of 6 % and 10 % for growth and for biosurfactant production (Fig 3C). From the data, the optimum inoculum concentration with OD reading of 0.545 ± 0.028 and optimum biosurfactant production with E-24 value of 25 ± 1.41 % were obtained. The result on the effect of incubation time on growth and biosurfactant production is presented in Fig 3D. The effect of different pH values on growth and biosurfactant production showed the optimum pH values to be 6 and 2, respectively and is presented in Fig 3E. Finally, Fig 3F shows that $NaNO_3$ and yeast extract favoured growth and biosurfactant production by the yeast isolates with OD value of 2.286 ± 0.01 and E_{24} value of 61.7 ± 3.53 %, respectively.



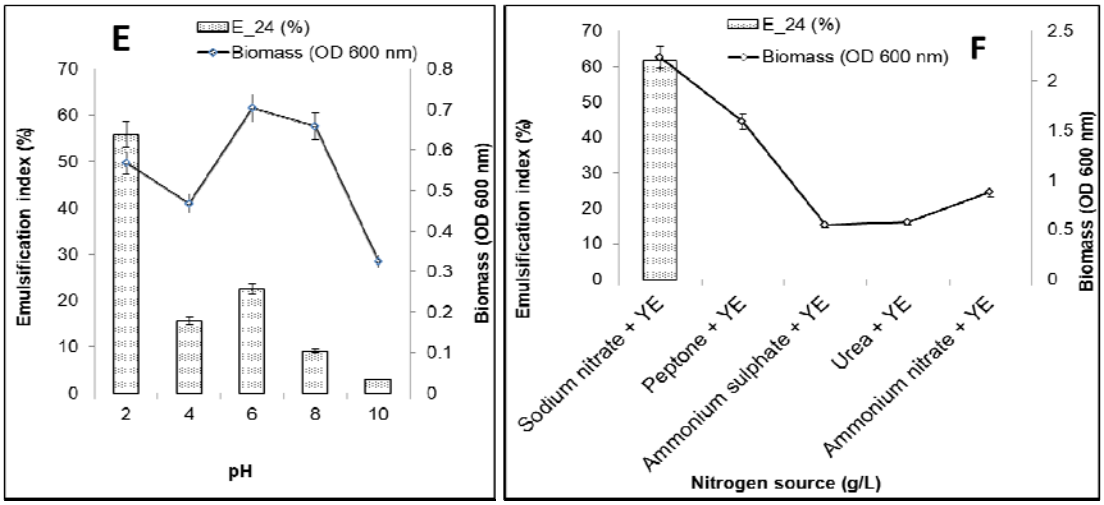


Figure 3: Effect of different cultural conditions on biomass and biosurfactant production. Legend: CC – Coconut chaff; **BB –** Beans bran; **Sba –** Soya bran; **SBb –** Sugarcane bagasse; **CP** – Cassava peel; **A –** Effect of different agro-wastes**; B -** Effect of different temperature**; C -** Effect of different inoculum concentration**; D –** Effect of different incubation time**; E –** Effect of different **pH; F –** Effect of different nitrogen sources.

3.6 Application of the Biosurfactant on Oil Emulsification

When the crude biosurfactant produced was applied on different oils, it showed varying degrees of emulsification (Fig 5).

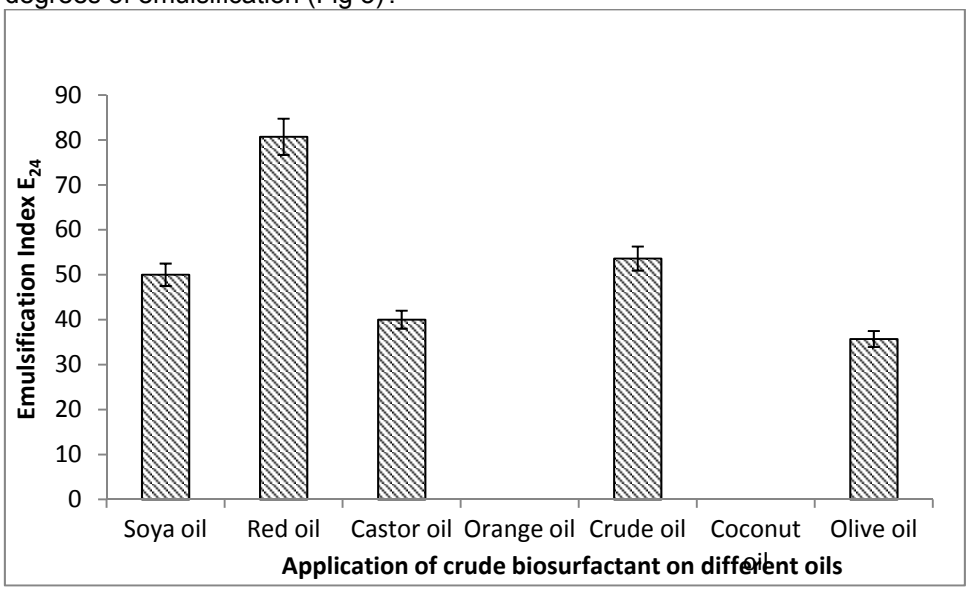


Figure 4: Application of crude biosurfactant from isolate SA2 on different oils

4. DISCUSSION

This study has demonstrated the ability of *Candida* sp., isolated from oil palm in producing biosurfactant. Although, there is dearth information available in literature, regarding the production of biosurfactant by yeasts isolated from oil palm. Konishi *et al*. [33] reported that biosurfactant-producing yeasts inhabit various vegetables and fruits. Iroha et al. [34] confirmed this by producing glycolipid biosurfactant from cashew fruit bagasse using *Pseudomonas aeruginosa.* Many researchers have reported that yeasts from different sources have the potentials of producing biosurfactants. Amaral *et al*. [35], reported that the majority of microbial biosurfactants are of bacterial origin. However, the pathogenic nature of this producing organism, has limited the application of these compounds in food and pharmaceutical industries. The study of biosurfactant by yeast has been of immense importance, because of 'generally regarded as safe' (GRAS) status that most of the species present. This GRAS status means that the yeasts do not present pathogenic or toxic considerations, thus, enhancing the application of their products for industrial usage.

The use of efficient screening strategy is the major key to successful discovery of new biosurfactant producers [36]. The screening methods employed in this study were haemolytic assay, oil-spreading test, emulsification index (E_{24}), emulsification assay, and tilted glass slide test. These methods have been previously reported for the identification of biosurfactant-producing microorganisms such as bacteria and yeasts: haemolytic assay [37-38], oil spreading [31, 30, 39], emulsification index [40-42], emulsification assay [43], tilted glass slide [44-46]. The yeast isolates screened showed varying results for the different screening methods employed. The biosurfactant-producing yeasts were selected based on its competence in giving positive results to all the screening methods. According to Satpute *et al*. [45], the examples of qualitative screening techniques are haemolytic assay and tilted glass slide test, whereas that of the quantitative screening techniques are emulsification index and oil spreading test. The screening techniques used in this study, employed both qualitative and quantitative methods. The use of these techniques is similar to the report of Satpute *et al.* [45], who used the combination of oil spreading, drop collapse, tilted glass slide and emulsification index to select biosurfactant producers. Satpute *et al*. [45], suggested that a single method is not suitable to select all the biosurfactant-producing microorganisms, and recommended the combination of methods. In addition, Dhimans *et al*. [47] used different screening methods, such as emulsification index, oil spreading method, oil displacement assay, surface tension measurement and drop collapse test to detect biosurfactant production. Ndibe and Usman [46], reported the confirmation of biosurfactant-production using the following classical techniques: haemolysis test, oil spreading, drop collapse, and emulsification index test.

To develop a process for maximum biosurfactants production is very crucial to optimize the medium and thus use suitable fermentation conditions. Incubation time has significant effects on biosurfactant production because microorganisms produce biosurfactant at different time intervals. This study investigated the effect of incubation time (24, 48, 72, 96, 120, 144 and 168 h) on the ability of the test yeast isolate to grow well (biomass formation), and produce biosurfactant. The optimum biosurfactant production with E_{24} value of 45 ± 7.071 was observed after 168 h (7 days) of incubation time. However, the optimum growth (1.720 ± 0.009) was also observed after 120 h (5 days) of incubation time. This is similar to the result of Cavalero and Cooper, [48] and Felsa *et al*. [49], who obtained maximum biosurfactant production from *Aspergillus ustus* after 5 days of incubation. Morita *et al.* (2006) reported that 16.3 gL^{-1} of glycolipid biosurfactants was produced by *Pseudozyma antarctica* after seven days of incubation using glycerol as a source of carbon. *Klebsiella pneumoniae* strain IVN 51 isolated from hydrocarbon polluted soil had optimum growth and biosurfactant production after five and two days of incubation, respectively [18].

Microbial processes are temperature dependent and, they usually get affected by change in temperature. According to Saharan *et al*. [50], most of the biosurfactant productions from fungi reported so far have been performed in a temperature range of 25 to 30 ^{o}C. It was observed that the growth of *Candida bombicola* reaches a maximum at temperature of 30 ^{o}

C, while 27 ^{o}C was the best temperature for the production of Sophorolipids [51] . This study is unique, in the sense that the yeast isolate was able to produce biosurfactant at an optimum temperature of 20 ^{o}C, with an E_{24} value of 54.7 ± 0.282 %, and biomass production with OD value of 1.965 ± 0.007 at optimum temperature of 30 ^{o}C. Khopade *et al*. [52], stated that many physiochemical factors such as pH, temperature, growth conditions and agitation have been shown to strongly influence microbial growth and metabolism. Among them pH of the production medium has proven to be the key factor for microbial growth.

The effect of pH (2, 4, 6, 8, 10) on the microbial growth and biosurfactant production were investigated. The results showed that maximum biosurfactant production was achieved at acidic pH of 2, with E_{24} value of 55.9 ± 2.85 % and the yeast isolates grew best at pH of 6 (0.703 ± 0.009). According to Bednarski *et al.* [53], the acidity of the production medium was the parameter studied in the synthesis of glycolipids by *Candida antarctica* and *Candida apicola*. When pH is maintained at 5.5, the production of glycolipids reached a maximum. The synthesis of the biosurfactant decreased without the pH control indicating the importance of maintaining it throughout the fermentation process. The pH of 6, favours the growth (biomass formation) and production of biosurfactant by *Pseudomonas aeruginosa* 2297, as reported by Kumar *et al*. [54]. *Candida lipolytica* at pH of 5.0 and *Candida batistae*, at pH of 6.0 produced maximum biosurfactant [55-56]. Amaral *et al.* [57], confirmed the production of Yansan, with a stable pH between 3 and 9 from *Yarrow lipolytica.*

It is estimated that substrate (carbon source) account for 10 to 30% of the total production costs of biosurfactant [58]. Thus, to reduce the cost involved in biosurfactant production, it is desirable to use low-cost raw materials like agro-industrial wastes. The effects of agro-industrial wastes (cassava peel, sugarcane bagasse, soya bran, coconut chaff, and beans bran) as carbon sources on biosurfactant production and growth of the yeast isolate was also investigated in this study. The result shows that cassava peel favoured the growth and production of biosurfactant with OD value of 1.884 ± 0.011 and E_{24} value of 64 ± 1.41 %, respectively. According to Nitschke *et al*. [59], microorganisms for biosurfactant productions can be selected using agro-industrial wastes such as cassava flour waste water. Nigeria has cassava in abundance, and most of the wastes are discarded. Therefore, finding industrial use for these wastes will have positive economic benefits.

Several nitrate salts such as sodium nitrate, ammonium nitrate, potassium nitrate was used as nitrogen sources for biosurfactant production. A combination of sodium nitrate and yeast extract were most influential nitrogen source. The result obtained revealed that these nitrogen sources favoured the growth of the test isolate with OD value of 1.884 ± 0.01, and E-24 value of 64 ± 1.41 % for biosurfactant production. The result is similar to the report of Abbasi *et al*. [60] that $NaNO_3$ (39.3 g) and yeast extract (3.93g) enhanced the optimum conditions for biosurfactant production by *Pseudomonas aeruginosa* MA01. Silva *et al*. [61], showed that *P. aeruginosa* 44T1 fail to give good biosurfactant yield with ammonium salts but instead gave good yield when $NaNO_3$. However, in another study, higher yield of biosurfactant by *Candida glabrata* UCP 1002 was observed with ammonium nitrate and yeast extract [62]. The effect of different inoculum concentration on the growth of the test isolate and for biosurfactant production was carried out. The result shows that inoculum concentration of 6 % (v/v) (E_{24} value of 25 ± 1.41 %) and 10 % (v/v) (OD value of 0.545 ± 0.007), enhanced the biosurfactant production and biomass formation by the test yeast isolate, respectively.

4. CONCLUSION

The results obtained from this study demonstrated the capacity of a yeast isolate from the sap of *Elaeis guineensis* to produce biosurfactant. The yeast isolate was identified as *Candida* sp. Production of biosurfactant from ecological safe source has an added advantage of excluding any risk of toxicity and pathogenic reactions to the environment. The ability to produce biosurfactant was dependent on the incubation media conditions.

Moreover, the biosurfactant was able to emulsify at varying degrees different hydrocarbons. Therefore, biosurfactant from *Candida* sp. can be scaled up for industrial production.

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COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

IVN and GCO conceived the study. IVN carried out the laboratory analysis. IVN, GCO, and CBC participated in the study design and coordination and drafting of the manuscript. All authors read and approved the final manuscript.

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