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Physicochemical screening of *Candida lusitaniae* P1 during synthesis of biosurfactant from coconut shell

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ABSTRACT

Aims: Fermentation and recovery are the major operating cost in biosurfactant production. Thus, the aim of this research was to synthesize biosurfactant from agricultural residues using suitable fermentation and recovery techniques in order to reduce the cost of production.
Methodology and results: Biosurfactant-producing yeast strains isolated from refined oil-contaminated soil samples using yeast extract-diesel agar (YEDA) were subjected to physicochemical screening such as drop collapse test, microplate analysis, oil spreading technique, emulsification index and thermostability. Based on the preliminary screening result, *Candida lusitaniae* P1, *C. parapsilosis* P51, *C. parapsilosis* D3 *and C. lusitaniae* E1 were selected for biosurfactant production using agricultural residue such as rice bran, wheat bran and coconut shell as substrate and crude supernatant was analyzed by gas chromatography mass spectrometry. *Candida lusitaniae* P1 strain produced 98.96 g/L of biosurfactant from coconut shell but when subjected to mutagenesis the yield decreased to 52.24 g/L.
Conclusion, significance, and impact of study: The physicochemical properties of biosurfactant produced using various carbon sources showed that coconut-shell is the best residue thus, variation in composition and concentration of biosurfactant obtained implies that the quality and quantity of biosurfactant produced depends on the carbon source and the genetic composition of the yeast isolate.

Keywords: Candida species, biosurfactant, drop collapse test, emulsification index, oil spreading technique

INTRODUCTION

Surfactants were originally made from renewable resources like fats and oils, whereas today, the majorities are of petrochemical origin (Albuquerque *et al.*, 2012). Biosurfactants are valuable microbial amphiphilic molecules with highly effective surface-active properties and useful in various biotechnology fields (Vyas and Dave, 2011). Biosurfactants have advantages over their chemicals counterparts because they are biodegradable, have low toxicity, are effective at extreme temperatures or pH values and show better environmental compatibility (Zheng *et al.*, 2012). The activity of biosurfactants depends on their structural components, e.g., the types of hydrophilic and hydrophobic groups and their spatial orientation (Bonmatin *et al.*, 1994). Biosurfactants are known to occur in a variety of chemical structures, such as glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids, and polymeric and particulate structures (Borjana *et al.*, 2002). The potential of these isolates in producing biosurfactant was examined by measuring the oil displacement and emulsification index during growth so as to know the extent of production of biosurfactant during different growth phase. The potential applications of biosurfactants in industries include emulsification and foaming for food processing, wetting and phase dispersion for cosmetics and textiles, or solubilization for agrochemicals. In addition, biosurfactants can be used in environmental applications such as bioremediation and dispersion of oil spills (Calvo *et al.*, 2004).

MATERIALS AND METHODS

Biosurfactant-producing yeasts isolation conditions

Biosurfactant-producing yeasts were isolated from refined crude oil-contaminated soil samples obtained from mechanic workshops in Osogbo, Osun state, Nigeria. Yeast extract diesel agar (YEDA) comprised of yeast extract agar (0.02 g/L) and membrane-filtered diesel oil (1%). The sterile yeast extract agar was allowed to cool to 45 °C before adding membrane-filtered diesel oil thus, utilized for isolation through spread plate technique at 30

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°C for 24 h. The isolates obtained were characterized to the species level using API 20 C AUX KIT (bioMérieux).

Preliminary screening of biosurfactant-producing yeast

Physiochemical characterization of biosurfactant-producing yeast

Biosurfactant production medium was prepared according to Abouseoud *et al.* (2007) with slight modification. The medium composed of mineral salt medium (g/L) [(Na_2HPO_4 (2.2); KH_2PO_4 (1.4); $MgSO_4 \cdot 7H_2O$ (0.6); $FeSO_4 \cdot 7H_2O$ (0.01); NaCl (0.05); $CaCl_2$ (0.02); yeast extract (0.020)]; carbon source (1% diesel), supplemented with 0.1 mL of trace elements solution of the following composition (g/L): [($ZnSO_4 \cdot 7H_2O$ (2.32); $MnSO_4 \cdot 4H_2O$ (1.78); H_3BO_3 (0.56); $CuSO_4 \cdot 5H_2O$ (1.0); $Na_2MoO_4 \cdot 2H_2O$ (0.39); $CoCl_2 \cdot 6H_2O$ (0.42); EDTA (1.0); $NiCl_2 \cdot 6H_2O$ (0.004); KI (0.66) and pH= 7.0 ± 0.2]. The medium was inoculated with selected biosurfactant producing yeasts and incubated on an orbital shaker at 150 rpm, 28 °C, pH 7.0 for 7 days (Hamzah *et al.*, 2013). The physiochemical properties of the samples such as drop collapse test, oil displacement test, microplate analysis, biosurfactant stability and emulsification index were determined according to the methods of Youssef *et al.* (2004) and Plaza *et al.* (2006) with slight modification using cell-free broth (crude supernatant) obtained by culture centrifugation at 5000 rpm for 20 min.

Drop collapse test

Sterile diesel oil of about 0.1mL was transferred onto the surface of 0.2 mL of crude supernatant transferred in a 96 well microplate. The occurrence of flat surface after 1 minute of adding the oil indicates positive result while beaded appearance (drop of oil remains beaded) indicates negative result (Plaza *et al.*, 2006).

Microplate analysis

Crude supernatant (0.2 mL) was transferred into a 96-microwell plate and viewed using a backing sheet of paper with grid. A positive result was recorded when there was no optical distortion of the grid (Chen *et al.*, 2007).

Oil spreading techniques

Sterile diesel oil (10 µL) was transferred onto the surface of 20 mL of distilled water in a sterile petri dish and 10 µL of crude supernatant was added respectively. The diameter of clear zone formed on the oil surface was measured and compared with the negative control (Distilled water and diesel oil, uninoculated medium) (Youssef *et al.*, 2004).

Thermostability analysis

Diesel oil (0.2 mL) was transferred onto the surface of 20 mL of distilled water and 0.2 mL of previously autoclaved supernatant at 121 °C for 30 min was added. The oil displacement area was measured. (Vilma *et al*., 2011).

Emulsification index (EI)

The EI of cell-free supernatant was determined by adding 1 mL of diesel oil to equal volume of crude supernatant, vortex-mixing for 2 min at 1800 rpm and leaving to stand for 24 h. The emulsification index is calculated as percentage of emulsified layer height (cm) divided by total height of the liquid column (cm) (Cooper and Goldenberg, 1987).

Chemical characteristics of biosurfactant

The biosurfactant was partially purified by adding 5 mL of crude supernatant and 2 mL of chilled acetone and kept at 4 °C overnight. The mixture was centrifuged at 3250 rpm for 20 min to obtain the precipitate, thus partially purified biosurfactant was extracted with 2 mL of isopropanol which later evaporated leaving behind an oil-like appearance. The chemical characteristics of biosurfactant produced was determined by TLC conducted on Silica gel plate using developing solvent system chloroform-methanol-water (65:25:4 v/v). After developing, the spots were revealed by saturated iodine steam and compared with the standards SDS and Tween 80. The retention factor (Rf) is calculated as distance travelled by the component divided by distance travelled by the solvent.

Mutagenesis of best biosurfactant-producing yeast using ultraviolet mutagen

The best biosurfactant-producing yeasts P1, P51, D3 and E1 selected based on the preliminary screening result were subcultured on yeast extract agar; exposed to physical mutagen (ultraviolet) for 30 min and incubated at 30 °C for 24 h in order to obtain mutant strains.

Microbial production of biosurfactant from agricultural residues

Agricultural residues utilized for production of biosurfactant are wheat bran, rice bran and coconut shell respectively. The production medium composed of mineral salt medium (g/L) [(Na_2HPO_4 (2.2); KH_2PO_4 (1.4); $MgSO_4 \cdot 7H_2O$ (0.6); $FeSO_4 \cdot 7H_2O$ (0.01); NaCl (0.05); $CaCl_2$ (0.02); Yeast extract (0.02)]; carbon source (2% agricultural residue), supplemented with 0.1 mL of trace elements solution of the following composition (g/L): [($ZnSO_4 \cdot 7H_2O$ (2.32); $MnSO_4 \cdot 4H_2O$ (1.78); H_3BO_3 (0.56); $CuSO_4 \cdot 5H_2O$ (1.0); $Na_2MoO_4 \cdot 2H_2O$ (0.39); $CoCl_2 \cdot 6H_2O$ (0.42); EDTA (1.0); $NiCl_2 \cdot 6H_2O$ (0.004); KI (0.66) and pH= 7.0 ± 0.2]. The best biosurfactant producing yeast isolates (both parent and mutant strains) based on their preliminary screening activity were inoculated into the medium separately and incubated on an orbital shaker at 30 °C and 150 rpm for 7 days. Crude supernatant was obtained through centrifugation at 5000 rpm for 20 min.

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The chemical characteristics of biosurfactant produced was determined by TLC conducted on Silica gel plate.

Extraction and quantification of biosurfactant using gas chromatography mass spectrometry

Biosurfactant extraction was done according to the methodology of Christova *et al.* (2004) with slight modification. The ionic strength (pH) of crude supernatant was reduced to 2 by acidifying with HCl and biosurfactant compounds were extracted by liquid-liquid extraction technique using 15 mL of diethyl ether. The extraction was repeated twice consecutively using equal volume of extractant; thus combining and concentrating the organic extracts on a rotary evaporator for analysis by gas chromatography – mass spectrometry HP 6890 Powered with HP Chemstation Rev.A 09.01 [1206] Software. The chromatography was done in HP-1 column (0.25 µm interior diameter × 30 m long) with a particle size of 0.25 µm using Selected Ion Mode (SIM) detector signal and nitrogen as the mobile phase at injection temp of 250 °C.

RESULTS

Colonial and microscopic morphology of biosurfactant producing-yeast isolates

Based on their colonial and biochemical characteritics, fifteen (15) biosurfactant-producing yeast isolates which include *Candida lusitaniae* (3 strains), *C. sphaerica* (3 strains), *C. rugosa* (2 strains), *C. parapsilosis* (4 strains), *Trichomonas asahii* (3 strains) were obtained (Table 1). The colonial morphology include colony shape (spherical or irregular shape), appearance (shiny or dull) and color (greyish or creamy) respectively. They also possessed the ability to utilize various sugars as their carbon source.

Table 1: Colonial and biochemical characteristics of biosurfactant producing-yeast isolates using API 20 C AUX kit.

Isolate code	Colonial Morphology (colony shape, appearance and color)	Biochemical Characteristics (Carbon assimilation)																			Identity
		GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF	
P1	Spherical, shiny and creamy	+	+	+	-	+	+	-	-	-	-	+	+	+	-	+	+	+	+	+	*C. lusitaniae*
P2	Spherical, shiny and creamy	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	+	+	-	+	*C. sphaerica*
P3	Spherical, dull and creamy	+	+	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	*C. rugosa*
P41	Spherical, dull and greyish	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	-	-	-	*T. asahii*
P42	Irregular, dull and greyish	+	-	-	-		-	-	+	-	+	-	-	+	-	+	+	+	-	-	*C. sphaerica*
P51	Spherical, dull, greyish	+	+	+	+	-	+	-	+	-	+	+	+	-	-	+	+	+	+	-	*C. parapsilosis*
P52	Irregular, dull and greyish	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+	-	-	*T. asahii*
P6	Spherical, shiny and creamy	+	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	*C. lusitaniae*
D1	Spherical, shiny and creamy	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	*C. parapsilosis*
D21	Spherical, dull, greyish	+	-	-	-	-	-	+	-	-	+	+	-	+	-	+	+	+	-	+	*C. sphaerica*
D22	Irregular, dull, greyish	+	+	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	*C. rugosa*
D3	Spherical, shiny and creamy	+	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	*C. parapsilosis*
KI	Spherical, shiny and creamy	+	+	+	+	+	+	-	+	-	+	-	+	-	-	+	+	-	+	-	*C. parapsilosis*
K2	Spherical, shiny and creamy	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	-	-	*T. asahii*
E1	Spherical, shiny and creamy	+	+	+	-	-	+	-	+	-	+	+	-	+	-	+	+	+	+	-	*C. lusitaniae*

P, Petrol soil sample; D, Diesel soil sample; K, Kerosene soil sample; E, Engine oil soil sample; GLU, D- Glucose; MDG, Methyl-D-glucopyranoside; GLY, Glycerol; NAG, N-acetyl-glucosamine; 2KG, Calcium-2-Keto-Gluconate; CEL, D-Celiobiose; ARA, L-ARAbinose; LAC, D-Lactose; XYL, D-Xylose; MAL, D-Maltose; ADO, Adonitol; SAC, D-Saccharose (sucrose); XLT, Xylitol; TRE, D-Trehalose; GAL, D-Galactose; MLZ, D-Melezitose; INO, Inositol; RAF, D-Raffinose; SOR, D-Sorbitol

Preliminary screening of biosurfactant-producing yeast

Among the fifteen biosurfactant-producing yeast subjected to preliminary screening using diesel oil as carbon source., crude supernatant obtained from five (5) yeast isolates namely *C. lusitaniae* P1*, C. parapsilosis* P51*, C. parapsilosis* D3*, C. sphaerica* P2 *and C. lusitaniae* E1 showed positive reaction to drop collapse test and microplate analysis. The crude supernatants also expressed high activity when subjected to oil spreading technique and thermostability analysis except *C. sphaerica* P2 supernatant that showed inactivity and instability towards oil spreading and thermostability analyses These crude supernatants also expressed wider emulsified layer with corresponding high emulsification index, which indicated the ability of the isolates to produce higher concentration of biosurfactant with retention factor (Rf) of 0.86-0.89 compared to other yeast isolates utilized (Table 2).

Mutagenesis of parent yeast isolates using UV mutagens

Candida lusitaniae P1, *C. parapsilosis* P51, *C. parapsilosis* D3 and *C. lusitaniae* E1 were selected as the best biosurfactant-producing isolates based on the preliminary screening and further subjected to

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mutagenesis. Among the crude supernatants obtained from the mutant fermentation media only *C. lusitaniae* P1, mutant showed best activities when subjected to physicochemical screening such as drop collapse test, microplate analysis, oil spreading technique, thermostability analysis and emulsification index respectively (Table 3).

Microbial production of biosurfactant from agricultural waste and quantification using gas chromatography mass spectrometry

Crude supernatant obtained from *C. lusitaniae* P1 parent and mutant strains using coconut shell as substrate showed best physicochemical properties compared to other agricultural residues and yeast isolates (Table 4). Quantification using GCMS also showed higher biosurfactant yield of 98.96749 g/L and 59.24385 g/L with variation in chemical composition respectively (Tables 5 and 6). These include Glycolipids (Rhamnolipid 1, Rhamnolipid 2, Rhamnolipid 3, Rhamnolipid 4 and Tetradecane); Fatty acid esters (Dodecanoic acid ethyl ester, Tetradecanoic acid ethyl ester and Hexadecanoic acid ethyl ester); Particulate (alpha-D-Glucopyranoside) and Lipopeptides (Polyoxyethylene docosyl ether and Polyoxypropylene docosyl ether).

Table 2: Preliminary screening of biosurfactant - producing yeast using diesel oil as substrate.

Yeast Isolates	Drop collapse test	Microplate Analysis	Oil spreading technique (cm)	Thermostability (cm)	Emulsification Index (%)	Rf
C. lusitaniae P1	+	+	1.8	1.6	65	0.89
C. sphaerica P2	+	+	0.0	0.0	40	0.80
C. rugosa P3	–	–	0.0	0.0	25	0.72
T. asahii P41	–	–	0.0	0.0	30	0.72
C. sphaerica P42	+	–	1.2	1.0	40	0.82
C. parapsilosis P51	+	+	1.6	1.4	50	0.86
T. asahii P52	–	–	0.0	0.0	30	0.74
C. lusitaniae P6	+	–	1.0	0.0	40	0.82
C. parapsilosis D1	–	–	0.0	0.0	25	0.72
C. sphaerica D21	–	–	0.0	0.0	20	0.70
C. rugosa D22	–	+	0.0	0.0	30	0.76
C. parapsilosis D3	+	+	1.8	1.2	60	0.86
C. lusitaniae K1	–	–	0.0	0.0	30	0.80
T. asahii K2	–	–	0.0	0.0	25	0.72
C. lusitaniae E1	+	+	1.6	1.2	55	0.86

Drop collapse test
–, Drop of diesel oil remains beaded (Biosurfactant absent)
+, Drop of diesel oil becomes flat (Biosurfactant produced)
Microplate analysis
+, No optical distortion of grid lines
–, Optical distortion of grid lines

Table 3: Physicochemical properties of biosufactant producing yeast mutant strains using diesel oil as substrate.

Yeast isolates	Drop collapse test	Microplate analysis	Oil spreading techniques	Emulsification Index (%EI)	Thermostability (cm)
C. lusitaniae P1m	+	+	1.2	45	1.0
C. parapsilosis P51m	+	+	1.0	30	0.8
C. parapsilosis D3m	–	+	0.8	20	0.6
C. lusitaniae E1m	+	–	1.0	25	0.6

m, mutant strains
Drop collapse test
–, Drop of diesel oil remains beaded (Biosurfactant absent)
+, Drop of diesel oil becomes flat (Biosurfactant produced)
Microplate analysis
+, No optical distortion of grid lines
–, Optical distortion of grid lines

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Table 4: Microbial production of biosurfactant from agricultural waste.

Physicochemical properties	Agriculture waste and yeast isolates											
	C. lusitaniae P1			*C. lusitaniae* P1m			*C. parapsilosis* P51			*C. parapsilosis* P51m		
	Wheat bran	Rice bran	Coconut shell	Wheat bran	Rice bran	Coconut shell	Wheat bran	Rice bran	Coconut shell	Wheat bran	Rice bran	Coconut shell
Drop collapse test	+	+	+	+	–	+	–	+	+	–	+	+
Microplate Analysis	+	–	+	+	–	+	–	+	+	+	+	+
Oils spreading technique (cm)	2.0	1.0	3.0	0.8	0.6	1.4	0.4	0.6	1.0	0.5	0.6	1.0
Emulsification Index (%)	50	45	60	50	30	55	30	40	45	40	45	50
Thermostability (cm)	1.4	1.2	1.8	0.6	0.4	0.8	0.2	0.4	0.6	0.2	0.4	0.8

Drop collapse test
–, Drop of diesel oil remain beaded (Biosurfactant absent)
+, Drop of diesel oil becomes flat (Biosurfactant produced)
Microplate analysis
+ = No optical distortion of grid lines
–, Optical distortion of grid lines

Table 5: GCMS analysis of biosurfactant obtained from coconut shell using *C. lusitaniae* P1 parent strain.

Retention time (min)	Area (pA*s)	Amount/Area	Amount (g/L)	Name	Group name
11.367	110.04016	4.90196e-1	53.94126	Rhamnolipid 1	Glycolipids
12.831	53.24825	4.40917e-1	23.47807	Rhamnolipid 2	“
14.503	60.09430	2.45038e-1	14.72539	Rhamnolipid 3	
15.854	21.23780	3.21254e-1	6.82273	Rhamnolipid 4	“
17.323	14.38736	3.76223e-7	5.41285e-6	Tetradecane	“
18.530	21.22846	3.82263e-7	8.11486e-6	Dodecanoic acid ethyl ester (Ethyl dodecanoate)	Fatty acid esters
19.782	17.25945	9.56096e-7	1.65017e-5	Tetradecanoic acid ethyl ester (Ethyl tetradecanoate or ethyl myristate)	“
20.946	20.17727	8.03678e-7	1.62160e-5	Hexadecanoic acid ethyl ester (Ethyl hexadecanoate or ethyl palmitate)	“
22.072	10.65461	1.02713e-7	1.09437e-6	Alpha-D-Glucopyranoside	Particulate
23.218	7.62481	3.74028e-7	2.85189e-6	Polyoxyethylene docosyl ether	Lipopeptides
25.322	3.03852e-1	8.03678e-7	2.44199e-7	Polyoxypropylene docosyl ether	“
Total	336.25632		98.96749		Biosurfactant

DISCUSSION

Microbial molecules which exhibit high surface and emulsifying activity are classified as biosurfactant. These molecules reduce surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures making them more potential agents for bioremediation (Banat *et al.*, 2000). Biosurfactants have advantages over their chemicals counterparts because of their superior properties which include high biodegradability, low toxicity, ecological acceptability, better environmental compatibility, salinity and stability (Velikonja *et al.*, 1993; Zheng *et al.*, 2012). The potential of the biosurfactant-producing yeast isolates was examined by measuring the oil displacement and emulsification index during growth in order to know the extent of production of biosurfactant during different growth phase. The drop collapse test and oil displacement tests are indicative of the surface and wetting activities (Youssef *et al.*, 2004). The oil displacement test is an indirect measurement of surface activity of a surfactant sample tested against oil; a larger diameter represents a higher surface activity of the testing solution (Tables 2-4) (Rodrigues *et al.*, 2006).

Drop collapse test assay (Tables 2-4) relies on the destabilization of liquid droplets by surfactants. In this research, drops of culture supernatant placed on an oil coated solid surface showed that liquid contains surfactants, the drops spread or even collapse (flat) because the force or interfacial tension between the liquid drop and the hydrophobic surface was reduced, while ab-

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Table 6: GCMS analysis of biosurfactant obtained from coconut shell using *C. lusitaniae* P1 mutant strain.

Retention time (min)	Area (pA*s)	Amount/Area	Amount (g/L)	Name	Group name
11.370	69.14611	4.90196e-1	33.89515	Rhamnolipid 1	Glycolipids
12.833	16.94531	4.40917e-1	14.50797	Rhamnolipid 2	"
14.506	18.21177	2.45038e-1	8.92297	Rhamnolipid 3	"
15.857	1.62625e-1	3.21254e-1	1.91775	Rhamnolipid 4	"
17.329	2.61337	3.76223e-7	9.83208e-7	Tetradecane	"
18.533	5.48556	3.82263e-7	2.09693e-6	Dodecanoic acid ethyl ester (Ethyl dodecanoate)	Fatty acid esters
19.785	5.59306	9.56096e-7	5.34751e-6	Tetradecanoic acid ethyl ester (Ethyl tetradecanoate or ethyl myristate)	"
20.951	6.36276	8.03678e-7	5.11361e-6	Hexadecanoic acid ethyl ester (Ethyl hexadecanoate or ethyl palmitate)	"
22.073	3.11208	1.02713e-7	3.19653e-7	Alpha-D-Glucopyranoside	Particulate
23.231	2.31935	3.74028e-7	8.67501e-7	Polyoxyethylene docosyl ether	Lipopeptides
25.261	3.79436e-2	8.03678e-7	3.04944e-8	Polyoxypropylene docosyl ether	"
Total	129.98994		59.24385		Biosurfactant

sences of biosurfactant in the supernatant resulted into repulsion of the polar water molecules from the hydrophobic surface and the drops remain stable (beaded). This implied that the stability of drops is dependent on surfactant concentration and correlates with surface and interfacial tension. Furthermore, involvement of biosurfactants in microbial adhesion and detachment from surfaces would prevent microbial biofilms formation in the food industry surfaces which are potential sources of contamination that may lead to food spoilage and transmission of disease (Hood and Zottola, 1995). Thus controlling the adherence is an essential step in providing safe and quality products to consumers.

The potential applications of biosurfactants in industries include emulsification and foaming for food processing, wetting and phase dispersion for cosmetics and textiles, or solubilization for agrochemicals, environmental applications such as bioremediation and dispersion of oil spills (Calvo *et al.*, 2004). Thus, the yeast isolates were investigated for their abilities to emulsify refined-crude oil (diesel oil) using Emulsification assay (Tables 2-4). The variation in emulsification index E24 % obtained from the different culture supernatant shows that the yeast isolates assimilated diesel oil source and produced bioemulsifier which either act through biosurfactant making cellular surface more hydrophobic or the biosurfactant enhanced aqueous solubilization and dispersion of the oil source. Temperature played an important role in the growth and biosurfactant production because the biosurfactant activity increased after being subjected to high temperature (121 °C) indicating that the biosurfactants are highly thermostable. (Tables 2-4).

The activity of biosurfactants depends on their structural components i.e. the types of hydrophilic and hydrophobic groups and their spatial orientation (Bonmatin *et al.*, 1994) and also exist in a variety of chemical structures, such as glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids, and polymeric and particulate structures (Borjana *et al.*, 2002). In this research, the crude supernatant was analyzed by GC-MSD in order to determine whether the biosurfactant produced contained multiple components or not. Thus, the variation in composition and concentration of different biosurfactant obtained implied that the compound contains glycolipids, fatty acids and polymeric structures. The physicochemical properties of biosurfactant screened (produced) using various carbon sources showed that coconut-shell is the best carbon source for biosurfactant production (Tables 5 and 6) which indicated that the quality and quantity of produced biosurfactant are affected and influenced by the nature of the carbon substrate (Cooper and Paddok, 1984).

CONCLUSION AND RECOMMENDATION

Candida lusitaniae P1 was the best producer of 98.97 g/L biosurfactant and even when subjected to mutagenesis, 59.24 g/L of biosurfactant was obtained using coconut shell. Thus, biosurfactant research should focus on the economics of biosurfactant production processes, such as utilization of inexpensive substrates as alternative low-cost fermentative media. Potential agricultural wastes (coconut shell) and best biosurfactant-producing strain (*C. lusitaniae* P1) isolated from petrol-contaminated soil are highly recommended.

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