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Applications of *Candida tropicalis* Bioactive Biosurfactant Produced Using Simple Substrate Medium

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BIOSURFACTANTS have been generating increasing interest due to their wide range of applications. This work aimed to propose a low-cost method for producing a biosurfactant using *Candida tropicalis* and studying its optimal conditions and applications.

A biosurfactant-producing *Candida* strain was selected and subjected to molecular identification. Optimum medium composition was determined using Plackett–Burman design; and response surface methodology using the central composite design. The Placket–Burman design showed that the maximum dry weight of biosurfactant (69.06mg/10mL) was obtained at the under optimal conditions of culture medium supplemented with 30g L^{-1} of carbon source and 1.5g L^{-1} of nitrogen source and incubation at 42˚C for 15 days. The extracted biosurfactant was characterized using FTIR. The dye decolorization/degradation ability was tested and antibacterial/antibiofilm assays were performed using the tissue culture plate method.

Crude biosurfactant of *Candida tropicalis* showed good antibacterial activity, with inhibition zones ranging from 1 to 2.8cm in diameter against standard bacterial strains. Using FTIR, the biosurfactant was confirmed to be sophorolipid. High degradation rates of 50.76% and 20.88% were recorded for methylene blue and Congo red dyes, respectively, using the partially purified biosurfactant, which was further, confirmed using FTIR analysis and HPLC. The partially purified biosurfactant showed significant anti-biofilm activity against pathogenic MDR *Klebsiella pneumoniae* biofilm at concentrations of 100 and 50mg mL^{-1}.

Conclusion: *Candida tropicalis* biosurfactant is potent at degrading different synthetic dyes in water, as well as exerting remarkable antibacterial and anti-biofilm activity against MDR pathogenic bacteria. Our results, suggest the value of using mixed substrates as low-cost substrates to increase the production of biosurfactant by *Candida tropicalis*.

Keywords: Anti-biofilm, Biosurfactant, *Candida tropicalis*, Dye degradation, Plackett–Burman design.

Introduction

Natural products have been considered sources of many medical agents due to their chemical structures and biological activities (Pham et al., 2019). Microorganisms that produce these natural compounds are now generating particular interest (Hara Kishore et al., 2007). Various microbial species are capable of producing a diverse range of natural compounds in the form of secondary metabolites, which can be important compounds for many applications in different fields (Garg et al., 2018).

Surfactants have been generated particular interest because of their range of applications in

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the manufacturing of industrial products as well as their application as emulsifiers (Shahwar et al., 2019; Suhail et al., 2019). Microbial biosurfactants have diverse chemical structures conferring great potential for biomedical, cosmetic, pharmaceutical, or food additive applications, although these applications require accurate characterization of possible toxic side effects (Saravanan & Vijayakuma, 2015).

Dyes are soluble organic compounds that exhibit high solubility in water, making them difficult to remove by conventional methods (Lellis et al., 2019). Biosurfactants have been used as promising agents in the field of bioremediation (Aulwar & Awasthi, 2016); their different forms are considered to make them useful for the bioremediation of contaminated soil, water, and other media (Bustamante et al., 2012; Karlapudi et al., 2018). Different *Candida* spp. are already known to produce biosurfactants (Priji et al., 2013; Garg et al., 2018). Sophorolipid (SL), a biosurfactant produced by *Candida* sp., has attracted particular attention; due to its antimicrobial activity, besides its role as an efficient spermicides, and use for anti-HIV drugs; and effective anti-cancer agents (Sleiman et al., 2009; Joshi-Navare & Prabhune, 2013). One advantage of SLs is that they have less irritating properties than conventional antibiotics (Kim et al., 2002). Aside from its clinical significance, only a few studies have reported on the exploration of *C. tropicalis* as a source of industrial biosurfactants (Ashish & Debnath, 2011). At present, the mechanism of action of biosurfactants is not well known, but they are hypothesized to exert activity of altering charge-charge properties, which may decrease the acquisition of antibiotic resistance by bacteria (Silveira et al., 2018). Therefore, the present study was established to propose a low-cost method for producing a biosurfactant using the yeast *Candida tropicalis*, studying the optimal conditions for the production of biosurfactant and assessing its environmental and medical applications.

Materials and Methods

Microbial strains

Three soil-isolated microbial strains that were defined as strong biosurfactant producers by biosurfactant tests (emulsification measurement, parafilm M test, lipase production, and drop collapsing test) (El-Shahed et al., 2020) and four MDR biofilm-producing clinical strains of *Klebsiella pneumoniae* previously characterized (Mohamed et al., 2020b) were used in this study. The standard pathogenic bacterial strains tested were as follows: *Escherichia coli* (ATCC 1922), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 25619), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 9144), and *Bacillus subtilis* (ATCC 19659).

Biosurfactant production

Antibacterial assay of partially purified biosurfactant

Partially purified biosurfactant produced by the three strains was subjected to antibacterial assays against standard pathogenic bacterial strains using the agar well diffusion method. Nutrient agar medium was prepared. Plates were swabbed with 100 µM of 0.5 McFarland bacterial cultures. Using a sterile well maker, wells were formed, to each of which 100 µM of biosurfactant was added. The plates were incubated at 37 °C for 24 h. The inhibited zone diameter was recorded (Garg et al., 2018).

Molecular identification

Molecular characterization of the *Candida* strain was performed using 18S rRNA gene sequencing, which was outsourced to Sigma Scientific Services Co; Cairo, Egypt (Abd-ElAzeem et al., 2019).

Optimization of growth medium for maximizing biosurfactant production using statistical analysis

Plackett–Burman design (Plackett& Burman, 1946)

An experiment was carried to determine the variables significantly influencing the dry weight of SLs (sophorolipids/biosurfactant). Twelve runs of experimental designs were applied to evaluate 11 variables, including three dummy factors (Table 1). Each variable was examined at two levels: –1 for the low level and +1 for the high level. The values of the two levels were set according to our previous preliminary experimental results and dummy factors, for which no values were assigned; they represent the integration of dummy variables into the experiment, allowing estimation of the experimental error of the effect. A total of 12 runs in the Plackett–Burman design were employed in duplicate and the average dry weight of biosurfactant was used as the experimental response of the design. Experiments were performed under static conditions in one batch culture.

TABLE 1 Levels of the factors tested in Plackett–Burman design

Symbol	Name	Units	Low (–1)	High (+1)
A	Carbon	gm L^{-1}	30	50
B	Nitrogen	gm L^{-1}	1	1.5
C	Temperature	˚C	35	42
D	Incubation time	Day	7	15
E	Yeast extract	Gm	0.99	1
F	Glucose	Gm	0.99	1
G	pH		6.99	7
H	Buffer solution	Ml	0.99	1
J	Dummy1		–1	1
K	Dummy2		–1	1
L	Dummy3		–1	1

Response surface methodology (RSM)

RSM has several classes of design, with different characteristics (Selvakumar et al., 2012). Central composite design (CCD) was used to optimize and identify optimal levels of the main variables. The optimal levels of the significant variables and the interactive effects of these variables on the dry weight of biosurfactants were analyzed by CCD (Table 2). In this study, the CCD design was applied to evaluate three factors at five levels with 14 runs were employed. Each of the tested factors (carbon source, nitrogen source, and incubation time) was assessed at five different levels, combining factorial points (−1, +1), axial points (−1, +1), and central point (0).

Identification of biosurfactant

The identification of biosurfactant was performed using Fourier-transform infrared spectroscopy (FTIR) analysis. The infrared spectrum of the sample was obtained using an FT-IR 4100 JASCO-Japan Fourier transform infrared spectrometer by the KBr pellet method in the wavelength range from 400 to 4000 cm^{-1}, with a resolution of $4cm^{-1}$ (Bakr et al., 2018).

Applications of biosurfactant

Environmental application

Percentage of dye decolorization: The percentage of dye decolorization or degradation was determined by the method of Guadie et al. (2017). Bi-fold dilution of 50mg mL^{-1} biosurfactant produced by *Candida tropicalis* was prepared. Fixed concentrations of 50mg L^{-1} of methylene blue and Congo red dyes were used. Tubes were incubated at two different temperatures 35 and 42˚C for 3 and 10 days. The dye decolorization experiment was carried out in distilled water, as synthetic wastewater contaminated with dyes. ELISA Reader was used to determine the decolorization rate using the following equation:

Decolorization rate= [Initial absorbance - Final absorbance / Initial absorbance] 100%.

The dye degradation rate was calculated using the following equation:

Sample Conc.= Sample area × St. Conc./ Area St.

Degradation (%)= (CO – Cf) / CO × 100

TABLE 2. The level of tested factors in the CCD (central composite experimental design)

Factors	Symbol	Level of factors				
		–2	–1	0	+1	+2
Carbon gm L^{-1}	**A**	28	30	40	50	52
Nitrogen gm L^{-1}	**B**	0.5	1.00	1.25	1.50	2
Incubation time /day	**C**	5	7	11	15	17

Degradation of dyes: A fixed concentration of dyes 50mg L^{-1} was used in this experiment; the three treatments were based on; addition of biosurfactant at 100mg mL^{-1}, addition of dead cells of *Candida tropicalis* at 100mg mL^{-1}, and addition of viable cells of *Candida tropicalis* at a concentration of 100mg mL^{-1}. The experiment was conducted in distilled water (as industrial wastewater contaminated with dyes). Regarding the experimental conditions of dye degradation, this experiment was performed at 42˚C under static conditions for 7 and 10 days. After the incubation period, an ELISA reader was used to measure dye degradation. Dye degradation by sophorolipid was confirmed using FTIR spectroscopy using the previously reported method.

HPLC analysis (Abd El-Kader et al., 2019) was carried out using high-performance liquid chromatography (HPLC) (Agilent Technologies 1260 Infinity II) equipped with a C18 column (150 × 4.6mm) with a flow rate of 0.9mL/min and absorbance at 590nm for Congo red dye, while methylene blue dye absorbance was at 668nm. The mobile phase of acetonitrile: water (60:40) was used as a solvent. Control samples of 50mg L^{-1} CR and MB dyes of methanol HPLC grade were used, while the treated samples were centrifuged at 3000rpm for 15min, after which 10mL of the supernatant was dissolved in 25mL of methanol. All samples were degassed in an ultrasonic water bath for 50min at room temperature, filtered through a syringe filter 0.45µM, and 20µL was injected.

Medical applications

Antibacterial activity: Antibacterial activity was evaluated using the well diffusion method (Garg et al., 2018). Muller Hinton Agar was poured into sterile plates; and, after it had solidified, 100 µl of the fresh culture of each *Klebsiella pneumonia* strain (0.5 McFarland) was swabbed. Wells were formed, to which 100µL of biosurfactant was added at concentration of 100mg mL^{-1}, followed by incubation at 37˚C for 18h. After the incubation, the diameters of the inhibition zones were measured.

Biofilm eradication

The effect of biosurfactant on the biofilm established for a period of 18 h was evaluated (Mohamed et al., 2020a). Concentrations of biosurfactant ranging from 0.3 to 100mg mL^{-1} were added to the wells, while negative control wells were left untreated. After 18h of incubation at 37˚C, wells were aspired, and plates were gently washed and finally stained as recommended by Mohamed et al. (2020a). The optical density (O.D.) was measured at 630nm using a microplate reader (STAT FAX 2100) in triplicate.

Statistical analysis

Optimization Data Analysis, Design-Expert Version 7.0.1 (Stat-Ease Inc., Minneapolis, MN, USA) was used for designing the experiments, regression, and graphical analysis of the data. The significance of the model and regression coefficients was determined using ANOVA.

The effects of different concentrations of *Candida tropicalis* biosurfactant on eradicating each bacterial strain were compared, and then data were analyzed by SPSS statistical software (version 12.0). Variables are expressed as mean ± standard deviation (SD).

Results and Discussion

Antibacterial activity of partially purified biosurfactants

Biosurfactants have many advantages and novel applications compared with chemically produced surfactants (Naughton et al., 2019). Different biosurfactant screening tests such as drop collapse test, oil spreading test, emulsification activity, and parafilm M test were performed and confirmed that the three active strains were able to produce biosurfactant (El-Shahed et al., 2020). These strains were biochemically identified as *Actinomycetes* spp., *Candida* spp., and *Pseudomonas* spp. in this study. Partially purified biosurfactant produced by the three strains was subjected to antibacterial assays against standard bacterial strains (Table 3).

Molecular identification of most potent biosurfactant producer

The most potent biosurfactant-producing strain with high activity was subjected to further molecular identification, and identified as *Candida tropicalis*, sharing 99.61% identity with the known nucleotide sequences of reference GenBank accession ID MN174072.1. A phylogenic tree was created, as shown in Fig.1, for *Candida tropicalis*, showing relationships among similar strains. Many species of *Candida* were reported to be good producers of biosurfactants, such as *Candida parapsilosis* (Garg et al., 2018), *Candida glabrata* (Asfora Sarubbo et al., 2006), *Candida lipolytica* (Rufino et al., 2007, 2011), *Candida sphaerica* (Sarubbo et al., 2013), and *Candida tropicalis* (Ankulkar & Chavan,

2019). Here, *Candida tropicalis* strain was chosen to study the production, identification, and further optimization of its biosurfactant, as well as study its applications.

Optimization of biosurfactant production

Optimization of the production of biosurfactant by *Candida tropicalis* was performed using a Plackett–Burman design, a screening design used for identifying significant variables among many potential variables (Korayem et al., 2015). Such statistical experimental designs are powerful tools for searching key variable rapidly as well as minimizing the error in determining the effect of the each category of the sources (Kalyani et al., 2014). The data presented in Table 4 indicate that biosurfactant dry weight varied from 16.1 to 69.06mg among the 12 runs (Figs. 2 and 3). To determine the significance of this model, analysis of variance was performed, as shown in Table 5. The results in the normal plot of the standardized effects from Plackett–Burman showed that A–carbon source, B–nitrogen source, C–temperature, D–incubation time, H–yeast extract, and dummy variables–J, K, and L had positive effects on the dry weight of the biosurfactant, whereas E–buffer solution, F–glucose, and G–pH adversely affected the dry weight. Statistically, only variables A, B, and D reached significance, so they were included in the CCD experiment step (Table 6). The maximum dry weight of biosurfactant/mg was achieved in run No. 9, reaching 69.06mg/ 10mL, which had the optimal conditions in the form of 30g L^{-1} of carbon source, 1.5g L^{-1} of nitrogen source, at 42°C for 15 days. The lowest dry weight of biosurfactant was observed in run No. 12, being 16.1mg/ 10mL.

TABLE 3. Antibacterial activity of crude microbial biosurfactant

Standard strains	Inhibition zone of crude microbial biosurfactant (cm)		
	S9(14)	S2(4)	S3(7)
E. coli 1922	1.00	1.30	1.05
E. coli 10536	1.10	1.60	1.40
Pseudomonas aeruginosa 25619	2.00	2.80	2.35
Pseudomonas aeruginosa 15442	1.90	2.20	1.90
Staphylococcus aureus 6538	1.85	2.35	1.95
Staphylococcus aureus 9144	2.00	2.50	2.10
Bacillus subtilis 19659	2.30	2.60	2.30

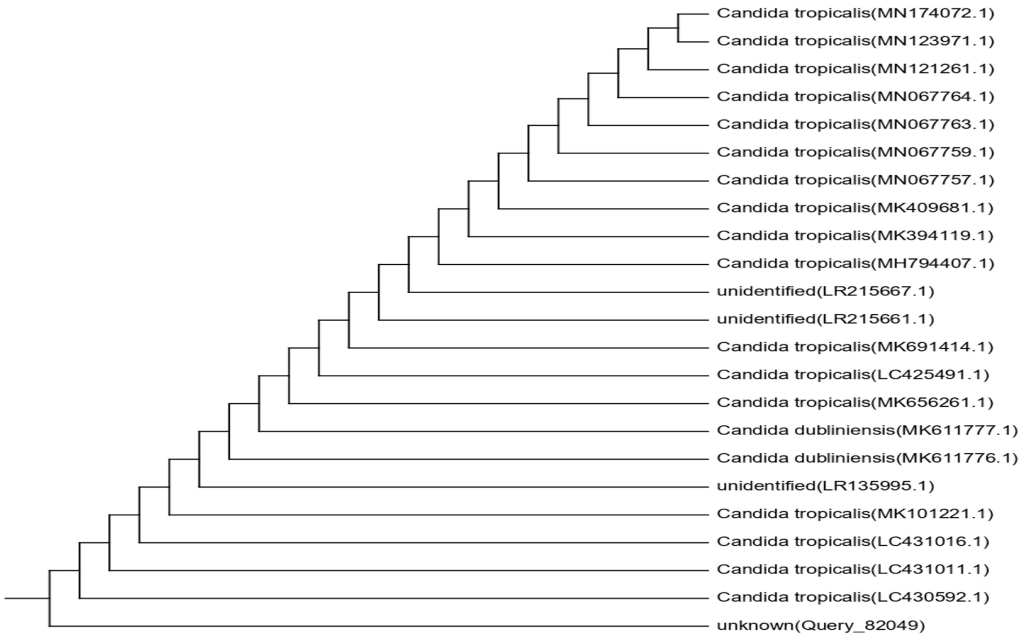

Fig. 1. Phylogenetic tree expressing the relationships of identified bacterial *Candida tropicalis* to similar bacteria based on the 16S rDNA sequences [The tree was clustered with the neighbor-joining method]

TABLE 4. Plackett-Burman design of variables (in coded levels) with a dry weight of biosurfactant as a response

Run	Experimental factors											Response
	A	B	C	D	E	F	G	H	J	K	L	Dry weight/mg
1	1	1	1	1	–1	1	1	1	–1	–1	–1	57.51
2	–1	–1	–1	1	–1	1	1	–1	1	1	1	47.81
3	–1	1	1	1	1	–1	1	1	1	–1	–1	42.5
4	–1	–1	1	–1	1	1	–1	1	1	1	–1	21.5
5	1	1	–1	–1	–1	1	–1	1	1	–1	1	23.7
6	1	1	–1	1	1	1	–1	–1	–1	1	–1	32.3
7	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	17.8
8	1	–1	–1	–1	1	–1	1	1	–1	1	1	19.6
9	–1	1	1	1	–1	–1	–1	1	–1	1	1	69.06
10	1	–1	–1	1	–1	–1	1	–1	1	1	–1	55.26
11	1	–1	1	–1	1	–1	–1	–1	1	–1	1	28.8
12	–1	1	1	–1	1	1	1	–1	–1	–1	1	16.1

TABLE 5. Analysis of variance of Plackett–Burman design

Source	Sum of squares	df	Mean square	F value	P value Prob>F	
Model	2616.36	3	872.12	7.62	0.0099	**Significant**
A-Carbon	0.48	1	0.48	4.196E-003	0.9499	
B-Nitrogen	6.90	1	6.90	0.060	0.8122	
D-incubation time	2404.20	1	2404.20	21.01	0.0018	
Residual	915.24	8	114.40			
Cor Total	3531.60	11				

*Significant at 5% level (P< 0.05), dF= Degree of freedom, P= Corresponding level of significance, F= Corresponding level of significance.

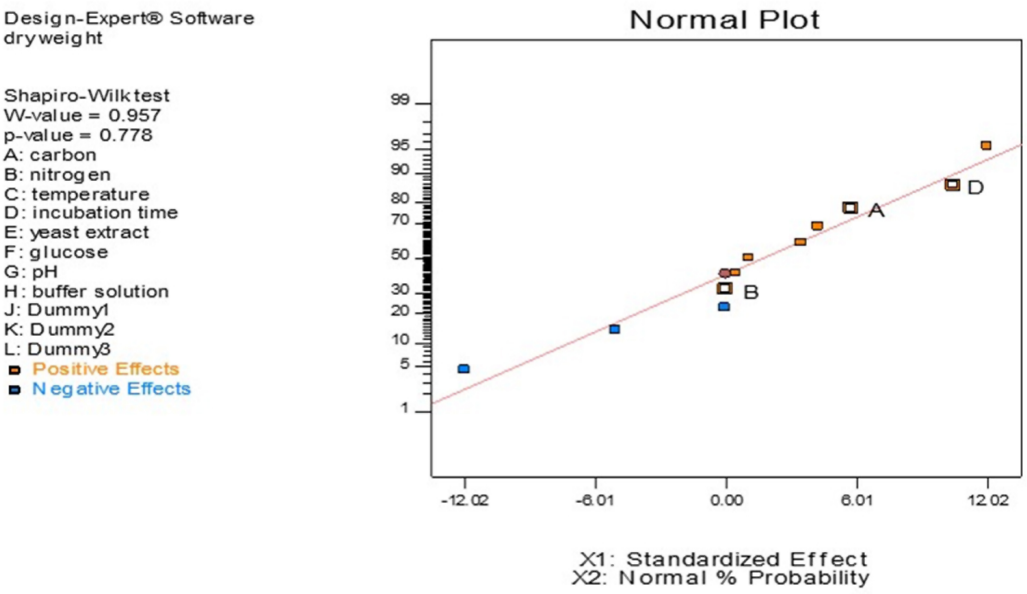

Fig. 2 .Normal probability plots showed positive factors and negative factors

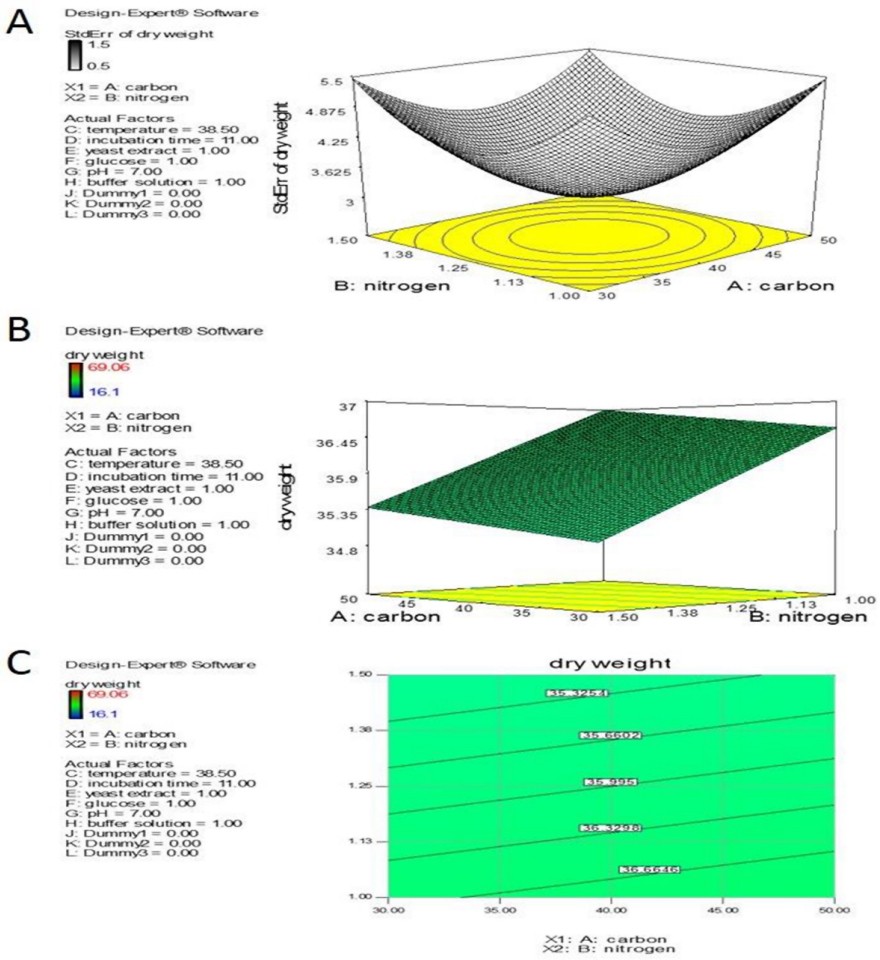

Fig. 3 Placket–Burman results: StdErr of dry weight (a), 3D Model (b), and contour model of dry weight (c)

TABLE 6. Effects of the variables and statistical analysis of the Plakett-Burman design

Factor	df	Coefficient	Standard error
Intercept	1	36.00	3.09
A-Carbon	1	0.20	3.09
B-Nitrogen	1	-0.80	3.27
D-incubation time	1	15.01	3.27
Std. Dev.	10.70		
Mean	35.99		
C.V.%	29.72		

*Std. Dev.= Standard Deviation, C.V.= Coefficient of variation. Pred- R^2 = 0.4698; Adj-R^2= 0.6437.

The results of the CCD design **(**Tables 7 and 8) showed that the most significant variable influencing dry weight was incubation time (Fig. 4). Run No. 14 showed the highest dry weight of 90.25mg/ 10mL. The optimum medium composition/condition observed was 40g L^{-1} of carbon source (a mixture of banana peel & date kernel,) and 1.25g L^{-1} of nitrogen source (a mixture of sodium nitrite and L-asparagine acid), at 42˚C for 17 days.

A confirmation experiment was performed manually by repeating the optimal run under optimal environmental conditions and an optimal nutritional medium. The expected/optimal run showed almost the same results; production of biosurfactant (dry weight) by the tested isolate was about 90.40mg/10mL.

TABLE 7. Experimental design and results of CCD

Run	X1	X2	X3	A dry weight of biosurfactant	
	Carbon source	Nitrogen source	Incubation time	Actual value	Predicated value
1	0	0	–2	31.51	25.74
2	1	0	–1	32.1	35.12
3	1	1	–1	40.75	39.28
4	1	–1	–1	30.66	30.96
5	–1	1	–1	35.91	29.40
6	0	–2	0	39	38.56
7	–2	0	0	35.5	37.53
8	2	0	0	43	50.58
9	0	2	0	45	49.54
10	–1	0	1	39	52.98
11	–1	1	1	51	57.14
12	1	–1	1	55.05	58.71
13	1	–1	1	57.95	58.71
14	0	0	2	90.25	62.36

TABLE 8. Analysis of response surface model CCD

Source	Sum of squares	df	Mean square	F value	P value prob> F	
Model	197.8	3	659.40	5.52	0.0169	**Significant**
A-carbon	214.48	1	214.48	1.79	0.2099	
B-nitrogen	127.80	1	127.80	1.07	0.3253	
D-incubation time	1965.30	1	1965.30	16.45	0.0023	
Residual	1194.24	10	119.42			
Cor Total	3172.44	13				
Std.Dev.	10.92		10.92			
Mean	44.76		44.76			
C.V.%	24.41		24.41			

*Pre R-squared = 0.3370, Adj-squared= 0.5106. The "Pre R-squared" of 0.3370 was reasonable agreement with the" Adj R-Squared" of 0.5106.

FTIR analysis of biosurfactant

Sophorolipid production was confirmed by FTIR analysis. The results showed a strong band at 3431.71cm^{-1} relating to the stretching O-H group, asymmetrical stretching of methylene group and methyl group (CH stretching vibration band; CH_2 & CH_3) at 2921.63cm^{-1}, unsaturated C=C bonds at 1627.63cm^{-1}, medium band indicating a lactone (C=O) group at 1733.69cm^{-1}, extending vibrations of carboxylic acids, aldehydes, and ketones at 1100.19cm^{-1}, an ester group at 1146.47cm^{-1}, and glucose indicating a sophorose moiety at 1025.94cm^{-1} (Fig. 5). These results are similar to those reporting that esters, acids, and lactones appeared at 1742cm^{-1}, along with a peak at 2900cm^{-1}, as well as a peak at 1000–1100cm^{-1} indicating an (O-C-O) group (Bajaj & Annapure, 2015; Elazzazy et al., 2015). The functional groups were similar to those in previously reported biosurfactants produced by various other *Candida* spp. (Verma et al., 2015; El-sheshtawy et al., 2016).

Degradation of different dyes determined using FTIR and HPLC

Concerning environmental applications, the results on the degradation of dyes as presented in Tables 9 and 10, showed that, for the two intervals, 10 days was the optimal incubation time in all treatments. Meanwhile, a higher degradation rate of 50.76% was achieved for methylene blue dye using the partially purified biosurfactant. FTIR spectroscopy was used to rapidly obtain information about the chemical compositions.

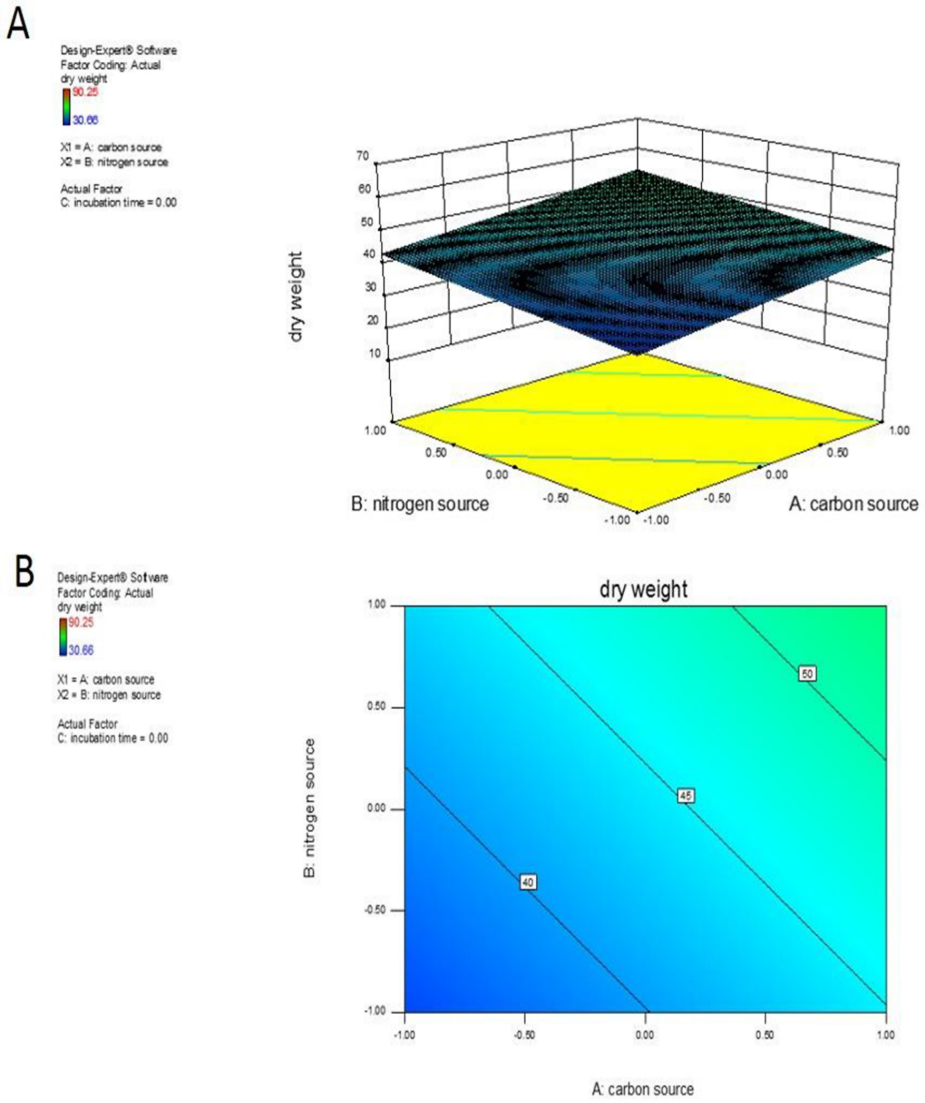

Fig. 4. CDD design: 3D Model (a), and contour model of dry weight (b).

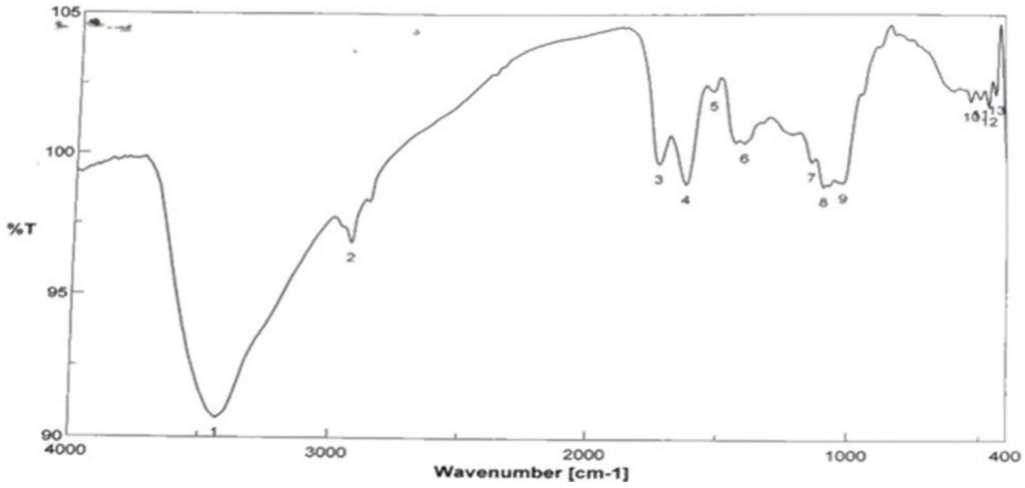

Fig. 5. FT-IR spectra show Biosurfactant (Sophorolipid)

TABLE 9. Biosurfactant decolorization percentages of different dyes at different temperature/incubation times

Biosurfactant concentration (mg mL^{-1})	Degradation percentages ± SD							
	3 days				10 days			
	Methylene blue		Congo red		Methylene blue		Congo red	
	37˚C	42˚C	37˚C	42˚C	37˚C	42˚C	37˚C	42˚C
50mg	17.82 ± 0.46	22.22 ± 7.25	56.09 ± 4.21	89.64 ± 1.12	26.46 ± 7.92	31.78 ± 0.46	92.93 ± 0.77	85.42 ± 1.75
25mg	15.73 ± 3.62	21.94 ± 2.615	51.94 ± 3.84	89.66 ± 0.87	19.61 ± 3.21	22.03 ± 0.71	92.72 ±1.18	89.26 ± 0.77
12.5mg	16.01 ± 5.49	17.05 ± 3.04	54.03 ± 2.76	88.45 ± 1.64	17.66 ± 1.06	21.15 ± 3.70	93.73 ± 0.45	84.51 ± 0.79
6.25mg	11.95 ± 1.95	10.46 ± 5.16	51.63 ± 4.16	36.64 ± 17.51	17.83 ± 3.21	14.88 ± 5.85	92.61 ± 0.82	86.62 ± 0.23

TABLE 10. Degradation of different dye at 42 ˚C using three Candida tropicalis treatment under static conditions

Dye used (50mg L^{-1})	Degradation percentages using different treatments ± SD					
	Biosurfactant		Dead cells		Viable cells	
	7 days	10 days	7 days	10 days	7 days	10 days
Congo red	18.284 ± 2.7675	20.884 ± 1.529	19.963 ± 4.1745	26.696± 0.89	24.473 ± 13.363	12.52 ± 5.998
Methylene blue	35.873± 1.627	50.761 ± 3.2615	17.189 ± 7.4674	38.839± 3.839	14.423 ± 0.283	26.355 ± 2.411

The FTIR spectra for CR dye and MB dye structures before and after the degradation process are shown in Fig. 6. Standard Congo red dye showed main functional groups such as an N-H stretching vibration of aromatic primary amine observed at 3464.49cm^{-1}, O-H stretching vibration at 2920.66cm^{-1}, strong S=O stretching sulfuric group (1061.62, 1177.33, and 1355.71cm^{-1}), C-N stretching vibrations at 1224.58cm^{-1} and 1122.37cm^{-1}, aromatic C=C stretching vibrations at 1447.31cm^{-1}, and azo group N=N stretching vibrations at 1583.27cm^{-1}. Meanwhile, upon treatment by sophorolipid, two peaks at different wavelengths (broad O-H stretching alcohol or medium N-H stretching primary amine at 3441.35cm^{-1}and medium C=N stretching at 1637.27cm^{-1}) were identified.

On the other hand, the FT-IR spectrum for the standard methylene blue dye featured benzene ring stretching corresponding to C=N and C=C vibrations at 1599.66 cm^{-1}, heterocycle skeleton at 1489.74, 1141.65, and 947.841cm^{-1}, aromatic amine groups at 1220.72cm^{-1}, aliphatic amine groups at 1248.68 and 1037.52cm^{-1}, methyl group symmetrical and asymmetrical bending vibrations of CH3 bonds at 1395.25cm^{-1}, and stretching vibrations of C-N terminal saturated dimethylamino groups and S=O stretching groups at 1177.33cm^{-1} before treatment. However, two peaks (broad medium N-H stretching primary amine at 3460.63cm^{-1} and medium C=N stretching at 1637.27cm^{-1}) were observed after sophorolipid treatment. According to the FT-IR spectra, the main functional groups of Congo red and methylene blue disappeared (Bartošová et al., 2017; Nasron et al., 2018), which is in agreement with a recent report describing that biosurfactant is effective for removing dye from aqueous solutions (Mahmoodabadi et al., 2019).

Generally, colored waters are among the most important hazards in industrial and environmental effluents (El-Sersy, 2007). Dyes are organic compounds that are highly soluble in water, making them difficult to remove by conventional methods (Lellis et al., 2019). From an industrial perspective, biosurfactants in their different forms are considered to be useful tools for the bioremediation of contaminated soil, water, and other media (Bustamante et al., 2012; Karlapudi et al., 2018). The use of biosurfactants was defined as being promising in the field of bioremediation (Aulwar & Awasthi, 2016), as they can enhance the bioremediation of hydrocarbons by increasing the substrate bioavailability for microorganisms, (Pacwa-Płociniczak et al., 2011) and increasing the surface hydrophobicity, allowing hydrophobic substrates to readily associate with microbial cells (Kaczorek et al., 2018).

The HPLC profile for the standard MB dye showed one peak at a retention time of 2.24 min with an area of 3896.082 (Fig. 7). While the samples treated by biosurfactant showed noise peaks indicating that complete degradation of MB may have occurred, treatment by viable cells of *Candida tropicalis* showed a peak at RT 2.45 min with an area of 9.888; finally, upon treatment with the dead cells of *Candida tropicalis*, a peak appeared at RT 2.458 min with an area of 18.327.

The HPLC profile for the standard CR dye showed one peak at a retention of time 1.114 min with an area of 1209.069 (Fig. 8). Meanwhile, the samples treated by biosurfactant showed one peak at RT 1.416 min with an area of 159.182. The samples treated with viable and dead cells of *Candida tropicalis* showed noise peaks, which indicated that complete degradation of CR may have occurred.

The HPLC results; indicated that methylene blue and Congo red dyes had been degraded by sophorolipid in 10 days, involving complete degradation for MB dye and 86.834% degradation for Congo red. Regarding the degradation of the MB and CR dye solutions by viable cells and dead cells of *Candida tropicalis*, there was complete degradation of CR by viable and dead cells, whereas for MB dye the degradation rates by viable and dead cells were 99.746% and 99.529 %, respectively. Our results are in line with some previous studies investigating the efficiency of biosurfactants/sophorolipids in dye degradation (Mnif et al., 2015). For example, immobilized *Candida tropicalis* was shown to reduce the level of Basic Violet and increase its rate of bioaccumulation. It was also reported that *Chlamydomonas* spp. and *Chlorella* spp. exhibited dye-removing capacity, with them having the ability to remove MB from aqueous solutions at a rate of 99.4% (Charumathi & Das, 2010; Al-fawwaz & Jacob, 2011). HPLC results also revealed that the rates of degradation by sophorolipid were 100% for MB and 86.834% for CR, suggesting the high emulsifying activity of biosurfactant (Ghribi et al., 2012) and demonstrating its enhancement of hydrocarbon biodegradation (Mnif et al., 2014).

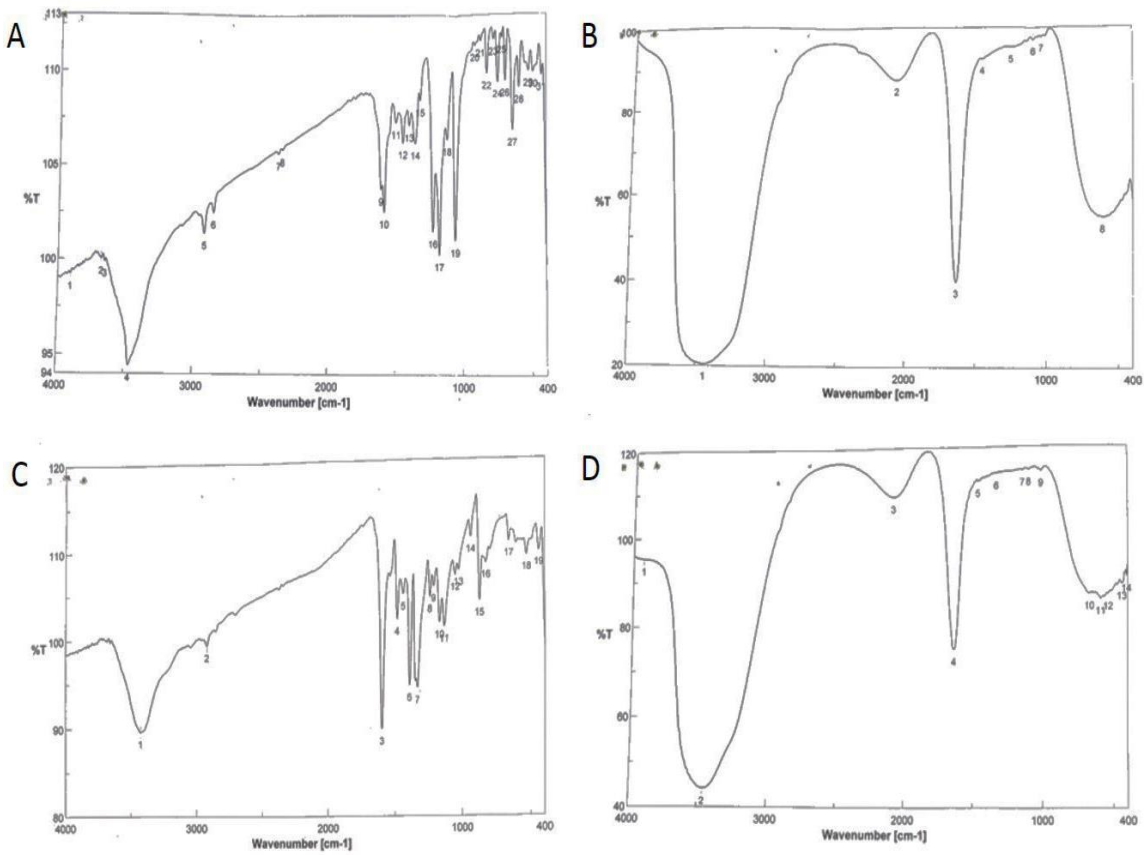

Fig. 6. Dye degradation confirmation using FTIR. A: Congo red standard, B: Treated water from Congo red by sophorolipid, C: Methylene blue standard, and D: Treated water from Methylene blue by sophorolipid

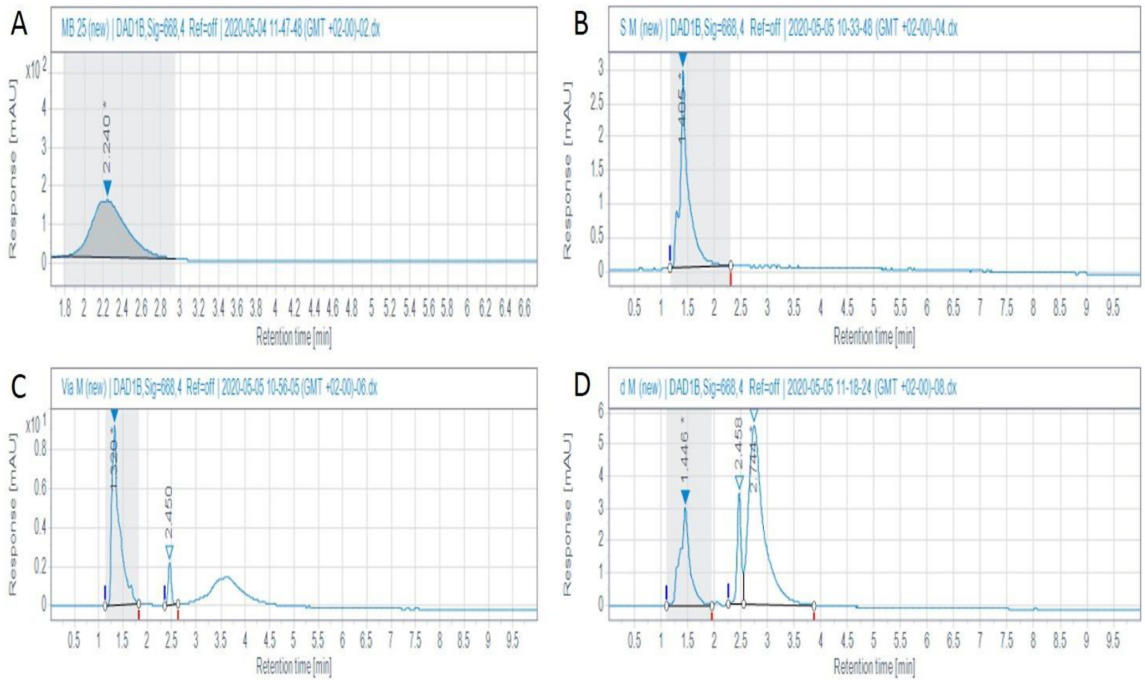

Fig. 7. HPLC chromatograph of methylene blue and Biodegradation. A: methylene blue (standard), B: methylene blue treated by biosurfactant, C: methylene blue treated by the viable cell of *Candida tropicalis*, and D: methylene blue treated by a dead cell of *Candida tropicalis*

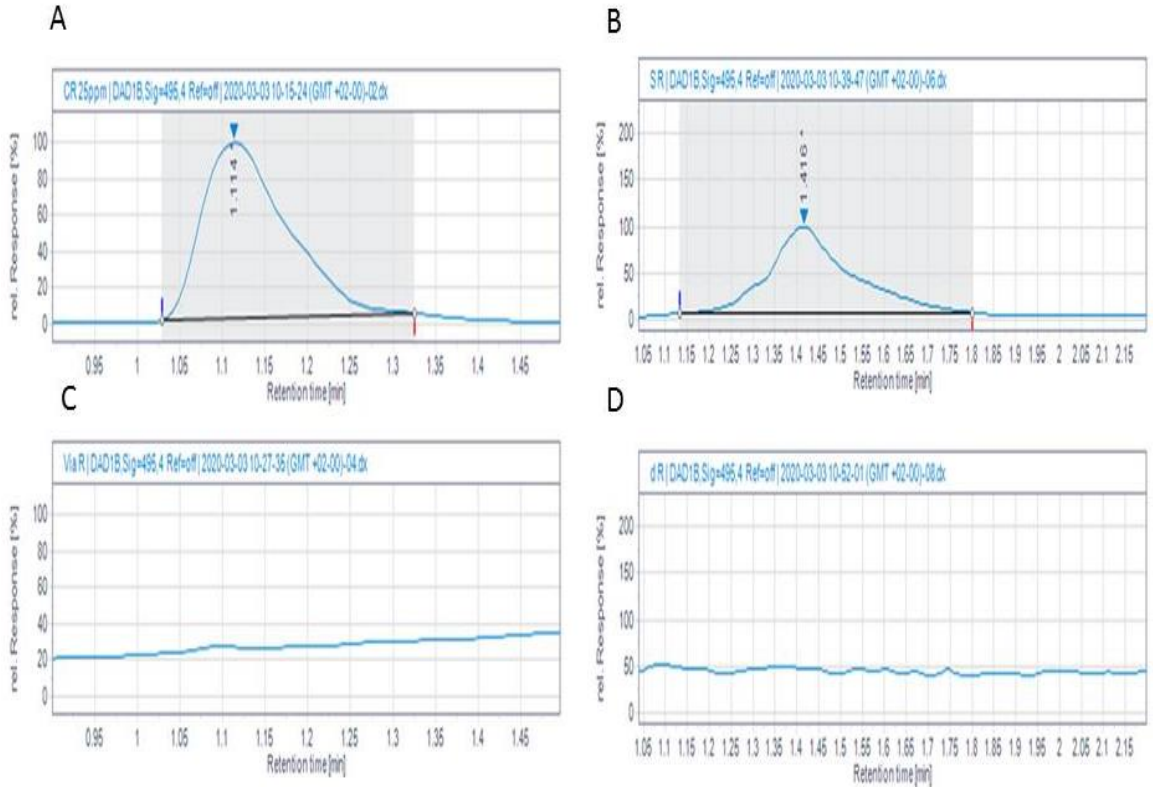

Fig. 8. HPLC chromatograph of Congo red and Biodegradation. A: Congo red (standard), B: Congo red treated by biosurfactant, C: Congo red treated by a viable cell of *Candida tropicalis*, and D: Congo red treated by a dead cell of *Candida tropicalis*

Antibacterial and anti-biofilm activity of biosurfactant

Bacterial biofilms are a critical problem, especially for pathogens that exhibit strong drug resistance (El-Shounya et al., 2019; Mohamed et al., 2020a). Here, the antibacterial and anti-biofilm effects of biosurfactant produced by *Candida tropicalis* were investigated on four MDR *Klebsiella pneumoniae* biofilm-producing pathogenic strains. No antibacterial activity was detected among the four used pathogenic strains, but biosurfactant produced by Candida *tropicalis* previously were showed activity on the standard strains of *P. aeruginosa, E. coli, S. aureus*, and *B. subtilis*. Other studies reported biosurfactant activities against pathogenic strains. *Candida parapsilosis* biosurfactant was reported to show significant antibacterial activity against pathogenic *E. coli* and *S. aureus* strains (Garg et al., 2018). The SLs of *Candida tropicalis* were also found to be highly effective against *S. aureus*, followed by *E. coli*; and *L. monocytogenes* standard strains (Ankulkar & Chavan, 2019), which is in agreement with our results on the standard strains. The antimicrobial activities of biosurfactants rely on different mechanisms, particularly the destruction of bacterial cells by directly disrupting the integrity of the plasma membrane or cell wall and affecting its permeability (Ndlovu et al., 2017; Kaczorek et al., 2018). On the other hand, the extracted biosurfactant in this study showed good anti-biofilm activity on the analyzed biofilms, with the best activity being achieved at concentrations of 100 and 50mg mL^{-1} (Fig. 9). Marked biofilm eradication at rates ranging from ≈7 to ≈23% was achieved using biosurfactant at a concentration of 100mg mL^{-1}, followed by eradication rates of ≈5% to ≈17.5% at a concentration of 50mg mL^{-1}. The results showed that the eradication rates decreased with decreasing concentration. Numerous previous studies presented findings in line with our results, showing that biosurfactants inhibit biofilm formation by preventing the adhesion of microorganisms (Díaz De Rienzo et al., 2015; Satpute et al., 2019). Sophorolipids are microbe-produced compounds that are particularly abundant in yeast, which have been shown to be biocompatible; and safe for human use, and to have good capacity to disrupt microbial biofilms (Díaz De Rienzo et al., 2015; Irie et al., 2005). At present, the mechanism of action of biosurfactants on preformed biofilm is not well understood, but it is hypothesized that they exert generalized activity of altering charge-charge properties (Davey et al., 2003), which may minimize the likelihood of biofilm-forming bacteria acquiring antibiotic resistance due to spontaneous mutations.

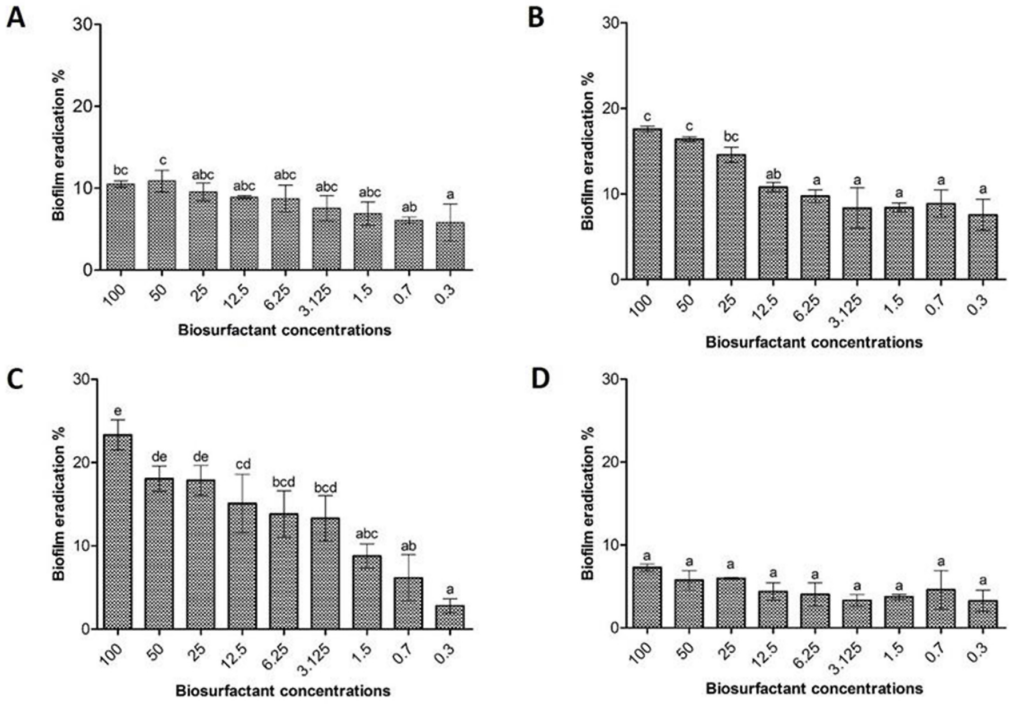

Fig. 9. Biofilm eradication percentages of *Candida tropicalis* biosurfactant against biofilm-producer *Klebsiella pneumoniae* strains at different concentrations, at which A: Kp1, B: Kp5, C: Kp8 and D: Kp9

Conclusion

According to the results of the Plackett–Burman design and statistical analysis in this study, it was concluded that, for the optimal production of biosurfactant by *Candida tropicalis*, obtained from Egyptian soil, ideal conditions include a mixture of banana peel & date kernel, as a carbon source, and a mixture of sodium nitrate and L-asparagine acid, as a nitrogen source, at 42˚C for 17 days. *Candida tropicalis* biosurfactant acted as a potent degrading agent for different synthetic dyes, as well as had exerting remarkable antibacterial and anti-biofilm activity against MDR pathogenic bacteria. Therefore, the used mixtures can effectively be consumed by *Candida tropicalis* strains as low-cost substrates to increase their biosurfactant production.

Abbreviations: CCD: Central composite design; CR: Congo red; MB: Methylene blue; MDR: Multi-drug resistance; RSM: Response surface methodology; SL: Sophorolipid.

Competing interests: The authors declare that they have no conflicts of interest.

Authors' contributions: M.Z.S., M.W.S. and M.I.B. were involved in conception of the research idea; and methodology design. M.M.S. El-Shahed; and S.H.M. carried out the laboratory work, interpreted the results, statistically analyzed the data, and prepared the article for publication. M.Z.S., M.W.S. and M.I.B. reviewed the article. All authors read and approved the final version of the article.

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تطبيقات مخفض التوتر السطحي المنتج من *Candida tropicalis* باستخدام ركائز وسط غذائي بسيط

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تولد المواد الخافضة للتوتر السطحي اهتمامًا متزايدًا بسبب تطبيقاتها الواسعه، ويهدف هذا العمل إلى اقتراح طريقة منخفضة التكلفة لإنتاج مخفض التوتر السطحى باستخدام *Candida tropicalis* ودراسة ظروفها وتطبيقاتها المثلى. تم اختياره وإخضاعه للتعريف الجزيئي. تم تحديد التركيبة المثلى للوسط الغذائي باستخدام تصميم بلاكيت-بورمان، ومنهجية سطح الاستجابة باستخدام التصميم المركب المركزي. أظهر تصميم بلاكيت-بورمان أنه تم الحصول على أقصى وزن جاف لمادة الخافضة للتوتر السطحي (69.06 ملجم/ 10 مل) في ظل الظروف المثلى لوسط الغذائي المضاف إليه 30 جرامًا من مصدر الكربون و1.5 جرامًا من مصدر النيتروجين والحضانة عند 42 درجة مئوية لمدة 15 يومًا. وأجريت الاختبارات المضادة للبكتيريا / المضادات الحيوية. أظهرت *Candida tropicalis* نشاطًا جيدًا مضادًا للبكتيريا، حيث تتراوح مناطق التثبيط من 1 إلى 2.8 سم ضد السلالات البكتيرية. باستخدام FTIR، تم التأكد من أن المادة الخافضة للتوتر السطحي هي sophorolipid. تم تسجيل معدلات تحلل عالية بلغت 50.76٪ و20.88٪ لأصباغ الميثيلين الأزرق والكونغو الاحمر، على التوالي، باستخدام خافض التوتر السطحي المنقى جزئياً، والذي تم تأكيده باستخدام تحليل FTIR و HPLC. أظهر باستخدام خافض التوتر السطحي المنقى جزئيًا نشاطًا كبيرًا مضادًا للبيوفيلم ضد *Klebsiella pneumoniae* المقاومه للمضادات الحيويه بتركيزات 100 و 50 مجم مل $^{-1}$

الخلاصة:*Candida tropicalis* فعالة في تحطيم الأصباغ الاصطناعية المختلفة في الماء، بالإضافة إلى انها مضاد للجراثيم ومضاد رائع للبيوفيلم فى البكتيريا المسببة للأمراض. تشير نتائجنا إلى قيمة استخدام ركائز مختلطة كركائز منخفضة التكلفة لزيادة إنتاج الماده الخافضة للتوتر السطحي بواسطة *Candida tropicalis* .