Production of Biosurfactant from Certain *Candida* strains Under Special Conditions

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ABSTRACT: Special conditions were devised to improve the production of biosurfactant from three *Candida* strains viz. *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25. Those strains were selected out of 25 strains previously screened for some aspects of industrial applications. A high rate of oil displacement was obtained from *C. albicans* No. 13 after 8 days of incubation under pH 7 and at temperature 20 ^{o}C; results confirmed by confidence interval (95%) at (r = 0.98, 0.99, and 0.99, respectively. $P < 0.05$). A high yield of biosurfactant was obtained from a culture of *Candida* isolates using carbohydrate substrate as a carbon source; among carbohydrates sucrose enhanced the best biosurfactant production. The optimum sucrose concentration was 1.0 %. $(NH_4)_2SO_4$ was the best nitrogen source for biosurfactant production at a concentration of 12 %. Highest amount of biosurfactant was recorded by the addition of adenine followed by guanine, threonine, arginine and vitamin C. Statistically our data showed a high significant correlation between all nutritional, environmental factors investigated and biosurfactant production ($P < 0.05$ in all).

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1. INTRODUCTION

Biosurfactants are metabolites, generally secondary, that constitute a group of diverse compounds synthesized by a wide variety of microorganisms (bacteria, filamentous fungi and yeasts) (**Cameotra and Makkar, 2004; Nitschke *et al.*, 2005a; Lu *et al.*, 2007; Banat *et al.*, 2010**). In recent years, much attention has been directed towards biosurfactants due to their amazing broad-range functional properties and diverse synthetic capabilities of microorganisms. It is interesting that the yeast isolates grow efficiently on hydrocarbons particularly, diesel and accompanying such growth was the production of surface active molecule with potent antimicrobial properties (**Ilori *et al.*, 2008**). Several elements, media components and precursors are reported to affect the process of biosurfactant production and the final quantity and quality. Different elements, such as nitrogen, iron and manganese are reported to affect the yield of biosurfactants; for example, the limitation of nitrogen is reported to enhance biosurfactant production in *P. aeruginosa* BS-2 (**Dubey and Juwarkar, 2001**) and *Ustilago maydis* (**Hewald *et al.*, 2005**). Similarly, addition of iron and manganese to the culture medium was reported to increase the production of biosurfactant by *B. subtilis* (**Wei *et al.*, 2003**).

Carbon is a very essential component of media for microbial growth and different microorganisms that produce biosurfactant. **Navon-Venezia *et al.*, 1995** demonstrated that the biological activity of a bioemulsifier, Alasan produced by *Acinetobacter radioresistens* was higher when citrate was used as carbon source than when acetate or Tris-HCl buffer was used. With different bacterial isolates, *Pseudomonas mallei* and *Pseudomonas pseudomallei*, **Okoro *et al.*, 2002** advanced that combination of acetate and diesel seem to be the preferred carbon sources when compared with other carbon sources such as crude oil, olive oil, kerosine and diesel for optimum bioemulsifier production by these organisms.

Nitrogen sources also effect the production of bioemulsifier by microorganisms. Among the inorganic salts tested by **Desai and Banat, 1997**, ammonium salts and urea were the preferred nitrogen sources for optimum bioemulsifier production by *Athrobacter paraffineus*. Other authors such as **Okoro *et al.*, 2002**, **Moussa *et al.*, 2006**, **Namir *et al.*, 2009** and **Batista *et al.*, 2010** have equally demonstrated maximum production of bioemulsifier when ammonium ions were used as nitrogen sources. Other media constituents like phosphate and metal ions also influence the production of bioemulsifier by microorganisms. For instance, in the production of glycolipid bioemulsifier by *Pseudomonas aeruginosa*, it was discovered that using K_2HPO_4 gave 3 fold yield of glycolipid than what was obtained when KH_2PO_4 was used (**Ramana and Karanth, 1989**). Magnesium ions have also been shown to positively affect the process of emulsification (**Sifour *et al.*, 2005**). This

work aimed to optimization of cultural conditions for biosurfactant production by some *Candida* strains. Similarly, this trail of some experiments to get a high yield of biosurfactant produced by three *Candida* strains tentatively under definite conditions as initiative steps for industrial applications.

2. MATERIALS AND METHODS

2.1. Source of samples:

Twenty five *Candida* strains (Hmadan, 2005) were used in this study.

2.2. Media used:

Three media used for culturing:

2.2.1. Medium E (consists of three parts):

Basal medium contained (g/l): KH_2PO_4, 2.7; K_2HPO_4, 13.9; sucrose, 10; NaCl, 50; yeast extract, 0.5; and $NaNO_3$, 1.0. pH adjusted at 6.9. Solution A contained 25 g/l of $MgSO_4$; solution B contained 100 g/l of $(NH_4)_2SO_4$; and solution C contained (g/l): EDTA, 0.5; $MnSO_4.H_2O$, 3.0; NaCl, 1.0; $CaCl_2.2H_2O$, 0.1; $ZnSO_4.7H_2O$, 0.1; $FeSO_4.7H_2O$, 0.1; $CuSO_4.5H_2O$, 0.01; ALK $(SO_4)_2$, 0.01; $Na_2MoO_4.2H_2O$, 0.01; boric acid, 0.01; Na_2SeO_4, 0.005; $NiCl_2.6H_2O$, 0.003. Solutions A and B were separately autoclaved, while solution C was filter sterilized. After autoclaving, 10 ml each of solutions A, B and C were added to 11 ml of the basal medium (**Youssef *et al*., 2004**).

2.2.2. Mineral salt medium (MSM):

It contained 2% glucose as the sole carbon and energy source. The MSM was a mixture of solution A and solution B. Solution A contained (g/l): $NaNO_3$, 2.5; $MgSO_4.7H_2O$, 0.4; NaCl, 1.0; KCl, 1.0; $CaCl_2.2H_2O$, 0.05 and 10 ml of concentrated phosphoric acid (85%).This solution was adjusted to pH 7.2 with KOH pellets. Solution B contained (g/l): $FeSO_4.7H_2O$, 0.5; $ZnSO_4.7H_2O$, 1.5; $MnSO_4.H_2O$, 1.5; K_3BO_3, 0.3; $CuSO_4.5H_2O$, 0.15 and $Na_2MoO_4.2H_2O$, 0.1. One ml of solution B was added to one ml solution A to form the MSM (**Bodour *et al*., 2003**).

2.2.3. Nutrient broth (Oxoid) supplemented with 0.1% yeast extract (**Beal and Betts, 2000**).

2.3. Subculture:

All *Candida* strains were grown on the three media mentioned above with adding agar to select the most suitable medium for growth. Then all *Candida* strains were grown in the broth of the best medium by inoculating 100 µl of the *Candida* strain suspension into 100 ml of the broth in 250 ml Erlenmeyer flask then incubated with shaking (200 rpm) at 28°C for 7 days.

2.4. Determination of biosurfactant production:

The culture broth of each *Candida* strain collected and cells were removed by centrifugation at 8000 rpm for 5 min. in cooling centrifuge (Sigma Co.). The surface tension of the culture supernatant was measured by using a capillary tube.

2.5. Surface tension test:

Surface tension was measured using capillary tube method according to **Martin** (**1993**) and applying the formula:

$$T = f \times r \times g \times h$$

Where: T = Surface tension.
f = Density of the fluid (1000kg/m^3)
r = Radius of capillary tube in meter.
g = Acceleration gravity (9.81m)
h = Highness of fluid in the capillary tube in meter.

Surface tension was measured for all strains of *Candida*.

2.6. Oil displacement test:

For the oil displacement technique, 50 ml of distilled water was added to petri dish (9 cm diameter) followed by addition of 20 µl of petroleum crude oil to the surface of the water. Ten µl of broth culture was then added to the surface of oil (**Morikawa *et al.*, 2000**). The diameter of clear zone on the oil surface measured and this was related to the concentration of biosurfactant by using a standard curve prepared with a commercially available biosurfactant, surfactin (Sigma, St. Louis, MO.) at concentrations ranging from 50 to 2000 mg/l.

2.7. Optimization of biosurfactant production:

2.7.1. Effect of different incubation periods:

The selected *Candida* strains were allowed to grow in 50 ml of broth medium E. at pH 7.0 and incubated at temperature 28°C in shaking incubator for different incubation periods 2, 4, 6, 8, 10, and 12 days. At the end of incubation periods, cultures broths were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different incubation periods were subjected for oil displacement and surface tension tests.

2.7.2. Effect of different incubation temperatures:

Fifty ml sterile broth medium E adjusted at pH 7 after sterilization were inoculated with the selected *Candida* strains under study and incubated for 7 days at different temperatures; 20, 23, 28, 32, 35 and 37ºC. At the end of incubation period, cultures broths were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different incubation

temperatures were subjected for oil displacement and surface tension tests.

2.7.3. Effect of different pHs:

The initial pH values of the broth medium E were adjusted to cover a range of 5 to 9 before sterilization, then inoculation was carried out by the selected *Candida* strains under study and incubated at 28ºC for 7 days under shaking condition. At the end of incubation period, cultures broths were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different cultures of pHs were subjected for oil displacement and surface tension tests.

2.7.4. Effect of different NaCl concentrations:

Broth medium E was supplemented with different concentrations of NaCl 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 % (w/v) individually, then the media were sterilized, inoculated with the selected *Candida* strains under study and incubated for 7 days at 28°C in shaking incubator. At the end of incubation period, cultures broths were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different concentrations were subjected for oil displacement and surface tension tests.

2.7.5. Effect of different sucrose concentrations:

The selected *Candida* strains under study were tested for their growth in broth medium E fortified with different concentrations of sucrose, namely by: 0.5%, 1.0%, 1.5%, 2%, and 2.5% individually. The media were sterilized, inoculated and incubated at 28ºC for 7 days in shaking incubator. After incubation, cultures broths were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different concentrations were subjected for oil displacement and surface tension tests.

2.7.6. Effect of different $(NH_4)_2SO_4$ concentrations:

Broth medium E was supplemented with the following concentrations of $(NH_4)_2SO_4$: 4, 8, 12 and 15% (w/v) individually, then the media were autoclaved, inoculated with the selected *Candida* strains under study and incubated at 28ºC for 7 days in shaking incubator. After incubation, cultures broths were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different concentrations were subjected for oil displacement and surface tension tests.

2.7.7. Effect of some growth factors:

The selected *Candida* strains under study were tested for their growth in broth medium E fortified with different growth factors. The following vitamins, amino acids and nitrogenous bases were used with the given concentration according to auxotrophs of **Davis (1948)**: vitamin C (50µg/ml), threonine (10 mg/ml), arginine (10 mg/ml), adenine (5 mg/ml) and guanine (5 mg/ml). The media inoculated with the selected *Candida* strains under study and incubated at 28ºC for 7 days in shaking incubator. After incubation broth cultures were centrifuged at 8000 rpm for 5 min. and then discarded cells. Supernatant from different concentrations were subjected for oil displacement and surface tension tests.

2.8. Statistical analysis:

The statistical SPSS version 15 and Microsoft Excel 2003 were used in data analysis. The confidence interval used for all statistical analyses was 95%. P-values less than 0.05 were significant.

3. RESULTS

3.1 Biosurfactant production by *Candida* strains using broth medium E:

All *Candida* strains were grown in broth medium E as most suitable medium for growth and consequently biosurfactant production. Surface tension was only criterion for measuring biosurfactant production in culture supernatant of *Candida*. *Candida albicans* number 13 lowered surface tension of supernatant from 73 to 28.2 mN/m, followed by *C. famata* number 11 and *C. albicans* number 25 were recorded 33.8 mN/m, each. Alternatively, *C. parapsilosis* number 17 showed the highest value of surface tension 67.6 mN/m. Rests of results are presented in table (1). From the previous results it is clearly that *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 were considered the most convenient organisms for foregoing studies.

3.2 Effect of certain environmental conditions and nutritional requirements on the selected *Candida* strains:

Some environmental factors as well as nutritional requirements were investigated to optimize the conditions of biosurfactant production from *Candida* strains. The three most potent strains from previous experiment were chosen as promising in biosurfactant production. These were *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

3.2.1. Effect of certain environmental conditions on production of biosurfactant:

Environmental physical factors such as incubation periods, different degrees of pH and different temperatures were tested for such purpose.

Table (1): Surface tension of *Candida* strains in broth medium E.

Strain No.	Strain	Highness of fluid (mm)	Radius of capillary tube (mm)	Surface tension (mN/m)
1	*C. glabrata*	8	0.575	45.1
2	*C. glabrata*	7	"	39.4
3	*C. tropicalis*	9	"	50.8
4	*C. albicans*	9	"	50.8
5	*C. glabrata*	9	"	50.8
6	*C. tropicalis*	10	"	56.4
7	*C. glabrata*	8	"	45.1
8	*C. glabrata*	9	"	50.8
9	*C. glabrata*	7	"	39.4
10	*C. parapsilosis*	8	"	45.1
11	*C. famata*	6	"	33.8
12	*C. tropicalis*	7	"	39.4
13	*C. albicans*	5	"	28.2
14	*C. albicans*	7	"	39.4
15	*C. famata*	7	"	39.4
16	*C. albicans*	9	"	50.8
17	*C. parapsilosis*	12	"	67.6
18	*C. famata*	10	"	56.4
19	*C. tropicalis*	9	"	50.8
20	*C. parapsilosis*	10	"	56.4
21	*C. famata*	8	"	45.1
22	*C. famata*	7	"	39.4
23	*C. albicans*	7	"	39.4
24	*C. parapsilosis*	8	"	45.1
25	*C. albicans*	6	"	33.8

3.2.1.1 Effect of different incubation periods:

Three *Candida* strains were cultivated for different incubation periods 2, 4, 6, 8, 10 and 12 days on broth medium E. The surface tension and oil displacement were estimated for each *Candida* strain at the incubation periods examined and the data are presented in table (2). Significant correlation was detected between the different incubation periods and oil displacement in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 *and C. albicans* No. 25 ($r = 0.97$, 0.98 and 0.99 respectively, $p < 0.05$ in all, Fig. 1). Similar conditions were observed for surface tension in the three *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 ($r = 0.94$, 0.99 and 0.95 respectively, $p < 0.05$ in all Fig. 2). The optimum incubation period observed for maximum production of biosurfactant was at 8 days by three tested *Candida* strains. The maximum production of biosurfactant was indicated by the results of oil displacement since they recorded 38, 41 and 36 mm for *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25, respectively.

Table (2): Effect of different incubation periods on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

Days	Oil displacement (mm)			Surface tension (mN/m)		
	C. famata No. 11	*C. albicans* No. 13	*C. albicans* No. 25	*C. famata* No. 11	*C. albicans* No. 13	*C. albicans* No. 25
2	28	32	29	50.8	45.1	45.1
4	30	35	32	45.1	39.4	45.1
6	35	38	34	45.1	33.8	39.4
8	38	41	36	39.4	28.2	39.4
10	36	39	35	39.4	28.2	39.4
12	34	37	32	45.1	33.8	45.1

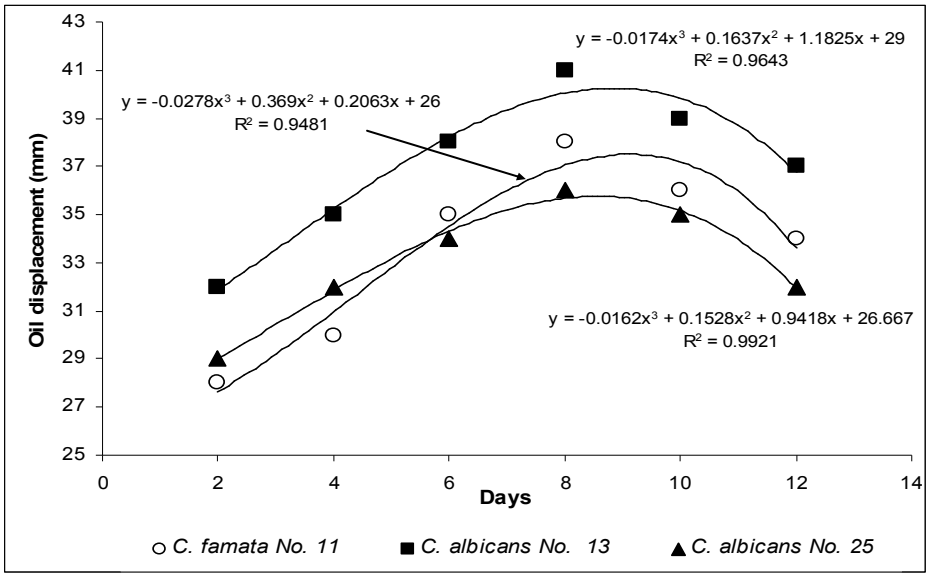

Fig. (1): Showing effect of different incubation periods on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

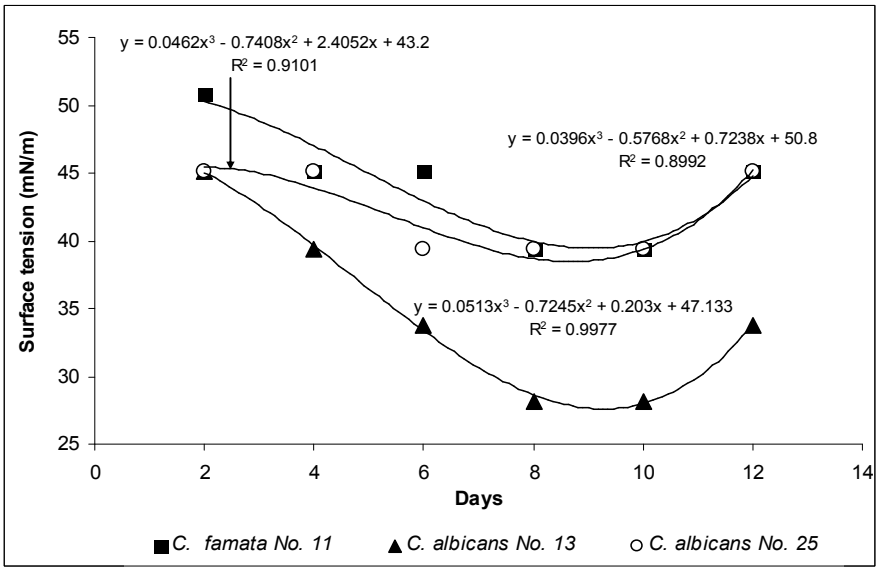

Fig. (2): Showing effect of different incubation periods on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.2.1.2 Effect of different pH values:

Surface tension and oil displacement of the three *Candida* strains under investigation in different pH values were examined in broth medium E. The ability of 3 tested *Candida* strains for production of biosurfactant in pH 7 was high, table (3). Significant correlation was detected between pH values and oil displacement in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 *and C. albicans* No. 25 ($r = 0.91$, 0.99 and 0.90 respectively, $p < 0.05$ in all, Fig. 3). Similar conditions were observed for surface tension in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 ($r = 0.90$, 0.95 and 0.95 respectively, $p < 0.05$ in all Fig. 4). Obviously, pH 7 was the optimum one for production of biosurfactant. *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 recorded 35, 42 and 32 mm oil displacement and 39.4, 28.2 and 39.4mN/m surface tension.

Table (3): Effect of different pH values on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

pH	Oil displacement (mm)			Surface tension (mN/m)		
	C. famata No. 11	*C. albicans* No. 13	*C. albicans* No. 25	*C. famata* No. 11	*C. albicans* No. 13	*C. albicans* No. 25
5	27	31	25	45.1	33.8	50.8
6	26	40	24	45.1	28.2	45.1
7	35	42	32	39.4	28.2	39.4
8	31	37	28	45.1	39.4	45.1
9	23	27	21	50.8	39.4	50.8

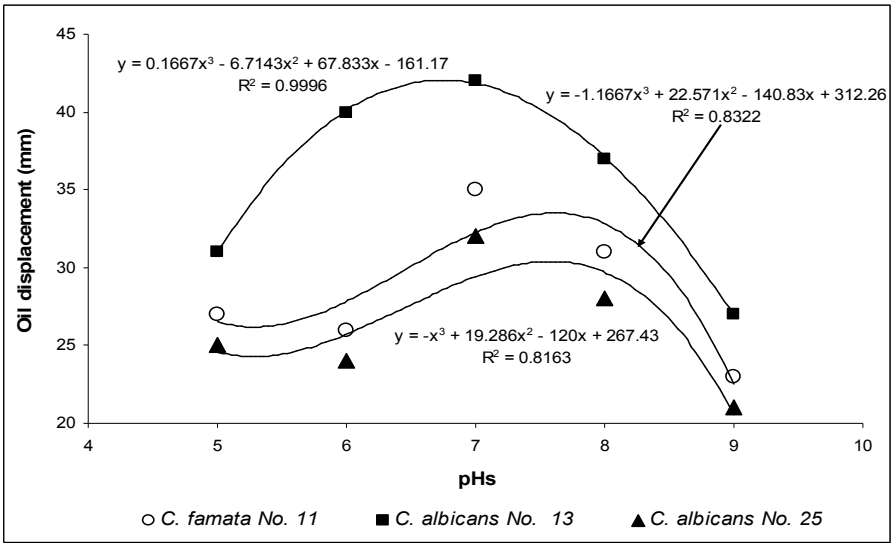

Fig. (3): Showing effect of different pH values on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

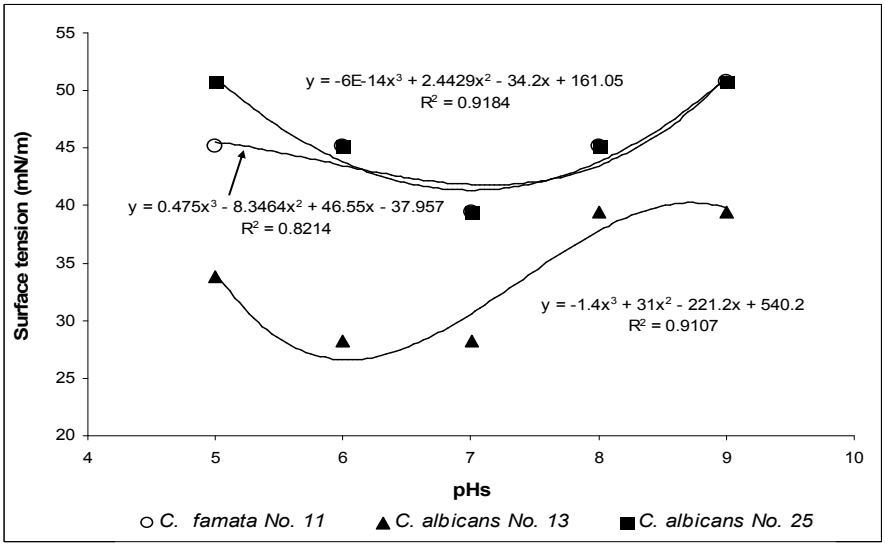

Fig. (4): Showing effect of different pH values on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.2.1.3. Effect of different incubation temperatures:

Effect of different temperatures on the production of biosurfactant by 3 selected *Candida* strains under investigation represented in table (4). Significant correlation was showed between the different incubation temperatures and oil displacement in the three *Candida* strains; *C. famata* No. 11, *C. albicans* No. 13 *and C. albicans* No. 25 ($r = 0.97$, 0.99 and 0.97, respectively, $p < 0.05$ in all, Fig. 5). Similar conditions were observed for surface tension in the three *Candida* strains; *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 ($r = 0.90$, 0.92 and 0.96 respectively, $p < 0.05$ in all Fig. 6). Data indicated that the optimum temperature for production of biosurfactant by all organisms was mostly 20°C. *Candida famata* No. 11 and *C. albicans* No. 13 showed 28 and 30 mm oil displacement, and 33.8 and 28.2 mN/m surface tension, respectively. It observed also that oil displacement inversely proportional and surface tension directly proportional to temperatures.

Table (4): Effect of different incubation temperatures on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

°C	Oil displacement (mm)			Surface tension (mN/m)		
	C. famata No. 11	*C. albicans* No. 13	*C. albicans* No. 25	*C. famata* No. 11	*C. albicans* No. 13	*C. albicans* No. 25
20	28	30	26	33.8	28.2	33.8
23	26	27	24	39.4	33.8	33.8
28	24	25	23	39.4	39.4	39.4
32	23	23	21	39.4	39.4	39.4
35	19	20	18	45.1	39.4	45.1
37	18	19	18	45.1	45.1	45.1

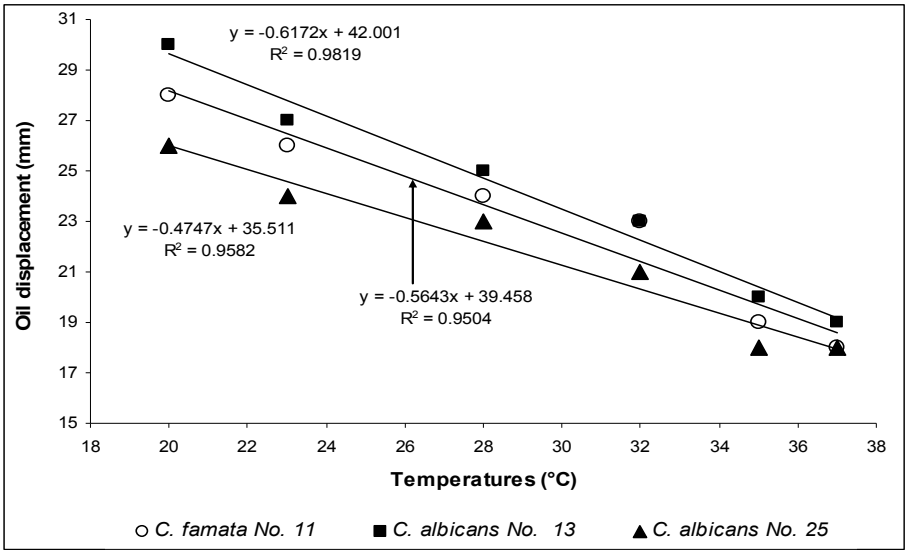

Fig. (5): Showing effect of different incubation temperatures on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

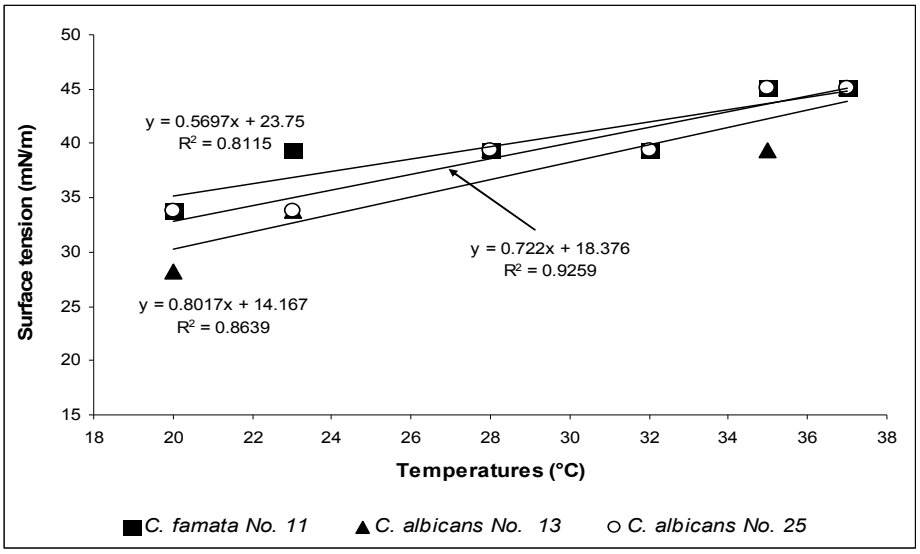

Fig. (6): Showing effect of different incubation temperatures on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.2.2 Effect of certain nutritional requirements on production of biosurfactant:

The best nutrient medium for the production of the biosurfactant agent by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 was medium E, so the study was carried out to investigate the best concentration of the best nutrients required by the organisms under study.

3.2.2.1 Effect of different NaCl concentrations:

The relation between salinity and biosurfactant production of 3 *Candida* strains under investigation was carried out and presented in table (5). Significant correlation was detected between NaCl concentratins and oil displacement in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 *and C. albicans* No. 25 ($r = 0.76$, 0.69 and 0.87 respectively, $p < 0.05$ in all, Fig. 7). Similar conditions were observed for surface tension in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 ($r = 0.92$, 0.64 and 0.82 respectively, $p < 0.05$ in all Fig. 8). The results of the current study revealed that growth of *Candida* observed till 10 % NaCl concentration supplemented in medium used. Data revealed that production of biosurfactant was increased as NaCl concentration increased that reached maximum at 5% and then decreased down to 10% NaCl. *Candida famata* No. 11 recorded fixed surface tension result (39.4 mN/m) at 2, 3, 4, 6 and 7% NaCl, and at concentration 1, 8 and 9 % recorded 45.1. Similarly, *C. albicans* No. 13 recorded fixed surface tension result (33.8 mN/m) at 1 and 2% NaCl, at 3, 6, 7 and 8% NaCl recorded 39.4 mN/m.

Table (5): Effect of different NaCl concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

NaCl (%)	Oil displacement (mm)			Surface tension (mN/m)		
	C. famata No. 11	*C. albicans* No. 13	*C. albicans* No. 25	*C. famata* No. 11	*C. albicans* No. 13	*C. albicans* No. 25
1	30	32	29	45.1	33.8	39.4
2	31	35	30	39.4	33.8	39.4
3	33	34	32	39.4	39.4	45.1
4	34	35	33	39.4	45.1	39.4
5	39	42	37	33.8	28.2	33.8
6	34	39	35	39.4	39.4	39.4
7	32	38	31	39.4	39.4	45.1
8	31	34	30	45.1	39.4	45.1
9	32	37	28	45.1	50.8	50.8
10	31	36	28	50.8	45.1	50.8

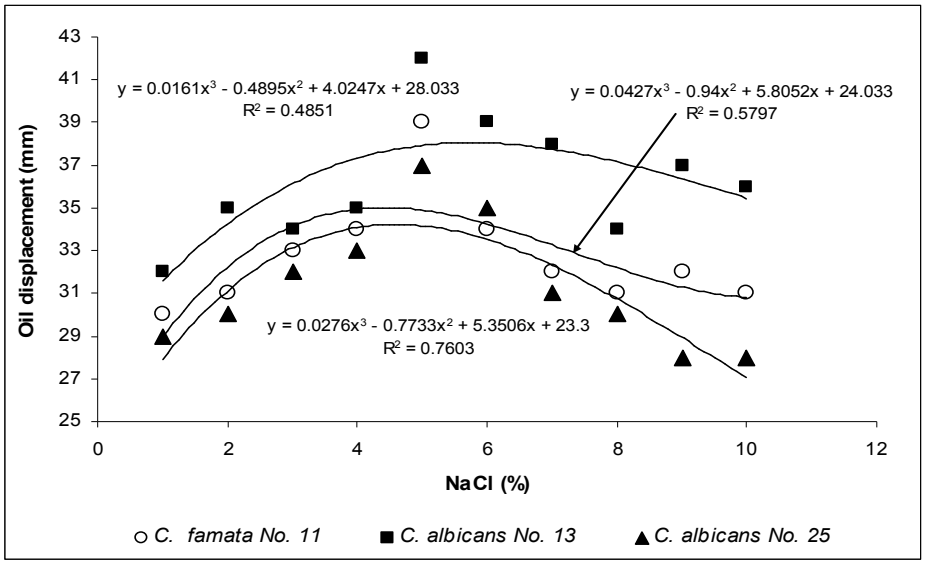

Fig. (7): Showing effect of different NaCl concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

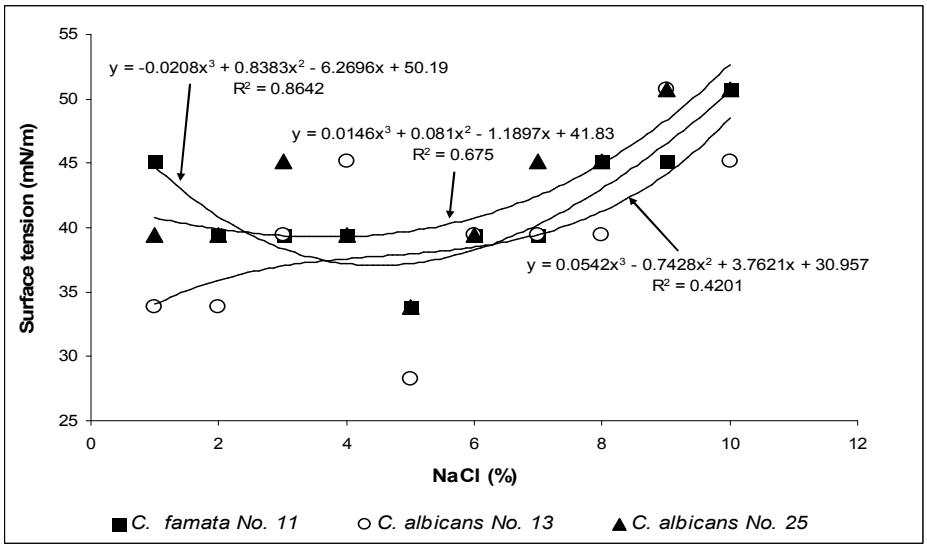

Fig. (8): Showing effect of different NaCl concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.2.2.2 Effect of different sucrose concentrations:

Three *Candida* strains were subcultured on different concentrations of sucrose. The investigated concentrations were 0.5, 1.0, 1.5, 2.0 and 2.5%. The concentration 1.0 % of sucrose was the optimum percentage for *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 to produce the maximum biosurfactant which indicated by oil displacement results (40, 42 and 38 mm) and surface tension results (33.8, 28.2 and 39.4 mN/m), respectively. All results are presented in table (6). Significant correlation was detected between sucrose concentrations and oil displacement in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 *and C. albicans* No. 25 ($r = 0.96$, 0.95 and 0.95 respectively, $p < 0.05$ in all, Fig. 9). Similar conditions were observed for surface tension in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 ($r = 0.90$, 0.98 and 0.90 respectively, $p < 0.05$ in all Fig. 10).

Table (6): Effect of different of sucrose concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

Sucrose (%)	Oil displacement (mm)			Surface tension (mN/m)		
	C. famata No. 11	*C. albicans* No. 13	*C. albicans* No. 25	*C. famata* No. 11	*C. albicans* No. 13	*C. albicans* No. 25
0.5	29	31	27	39.4	45.1	45.1
1.0	40	42	38	33.8	28.2	39.4
1.5	38	40	35	39.4	33.8	45.1
2.0	37	40	34	45.1	39.4	45.1
2.5	33	35	31	45.1	39.4	50.8

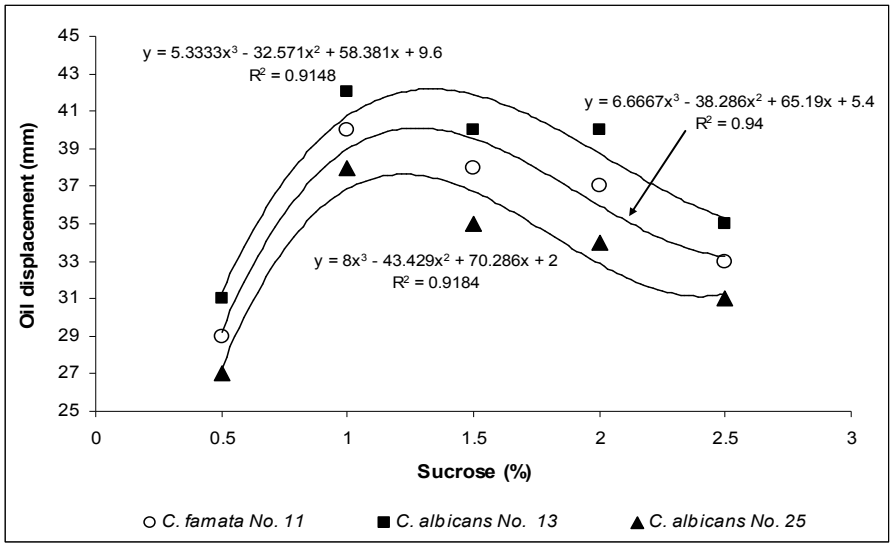

Fig. (9): Showing effect of different sucrose concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

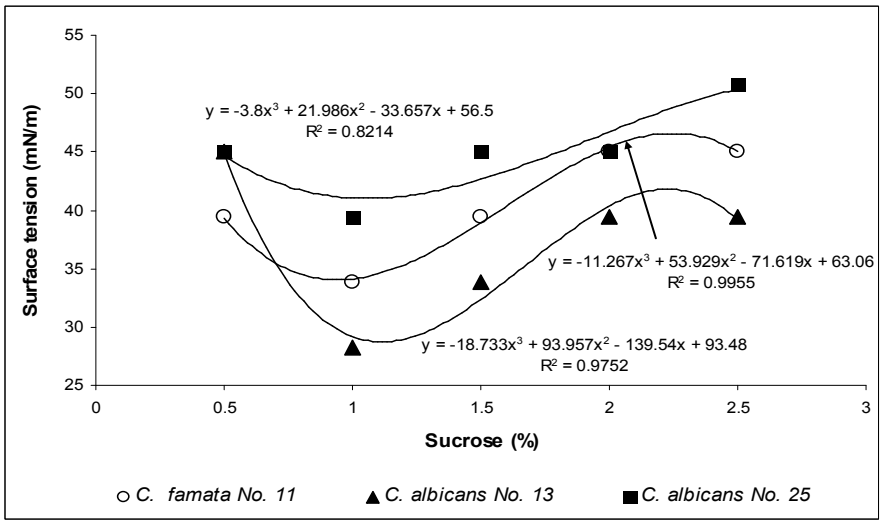

Fig. (10): Showing effect of different sucrose concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.2.2.3 Effect of different concentrations of $(NH_4)_2SO_4$:

Effect of different concentrations of $(NH_4)_2SO_4$ on biosurfactant production of 3 tested *Candida* strains are presented in table (7). Significant correlation was detected between $(NH_4)_2SO_4$ concentrations and oil displacement in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 *and C. albicans* No. 25 ($r = 0.94$, 0.94 and 0.99 respectively, $p < 0.05$ in all, Fig. 11). Similar conditions were observed for surface tension in the three *Candida* strain; *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 ($r = 0.99$, 0.98 and 0.98 respectively, $p < 0.05$ in all Fig. 12). Production of biosurfactant increased as concentration of $(NH_4)_2SO_4$ was increased till 12 % and then decreased. The optimum concentration of $(NH_4)_2SO_4$ was 12 % that recorded 33, 36 and 31 mm of oil displacement and 33.8, 28.2 and 39.4 mN/m of surface tension for *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25, respectively. However, a minimum percentage of $(NH_4)_2SO_4$ (4%) was still stimulate the organisms to produce biosurfactant, but to the lowest limit.

Table (7): Effect of different $(NH_4)_2SO_4$ concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

$(NH_4)_2SO_4$ (%)	Oil displacement (mm)			Surface tension (mN/m)		
	C. famata No. 11	*C. albicans* No. 13	*C. albicans* No. 25	*C. famata* No. 11	*C. albicans* No. 13	*C. albicans* No. 25
4	30	32	28	45.1	50.8	50.8
8	29	31	27	45.1	45.1	45.1
10	30	32	29	39.4	39.4	39.4
12	33	36	31	33.8	28.2	39.4
15	31	34	28	39.4	33.8	45.1

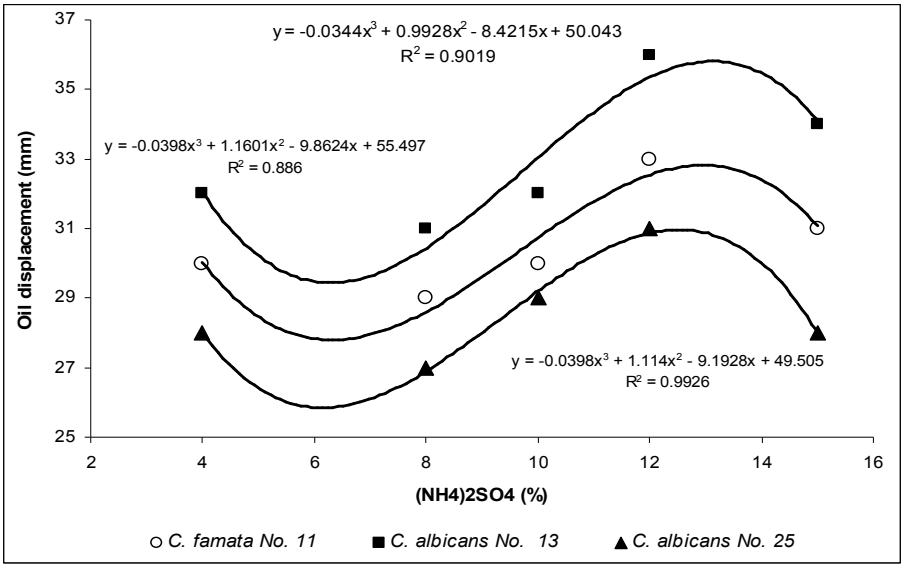

Fig. (11): Showing effect of different $(NH_4)_2SO_4$ concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

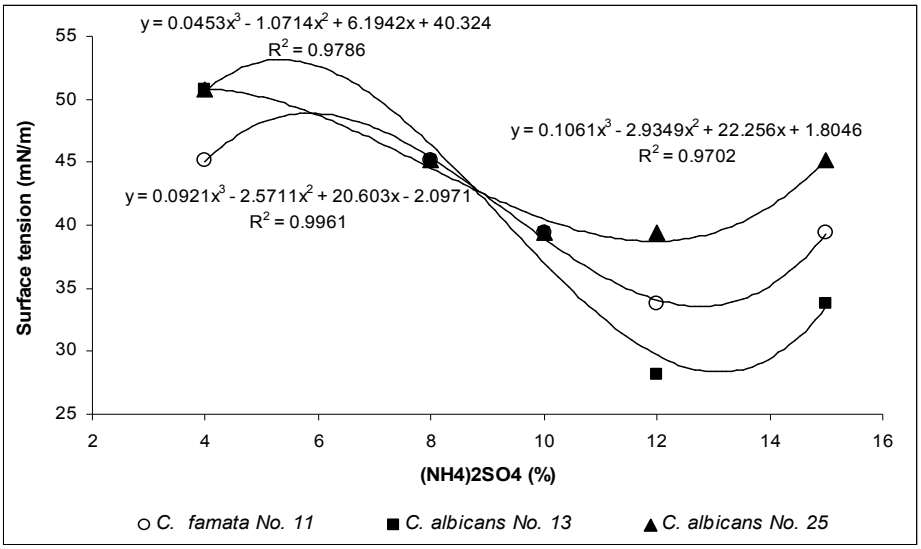

Fig. (12): Showing effect of different $(NH_4)_2SO_4$ concentrations (%) on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.2.2.4 Effect of some vitamins and amino acids:

Different types of growth factors could affect on biosurfactant production. The relation between biosurfactant production and some amino acids, vitamin and nitrogenous bases was carried out and presented in figures 13 and 14. Data showed that the maximum productivity of the biosurfactant was achieved by using adenine followed by guanine, threonine, arginine and vitamin C.

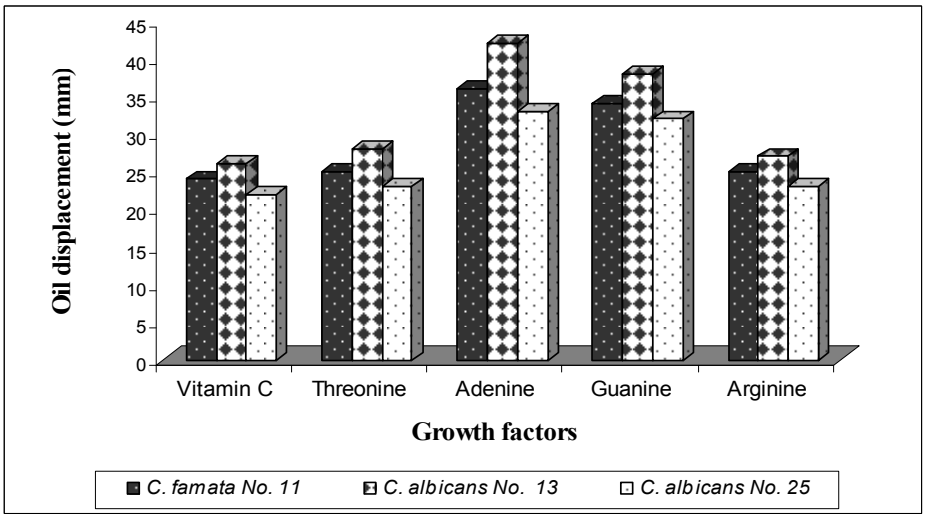

Fig. (13): Showing effect of different growth factors on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as oil displacement [mm]).

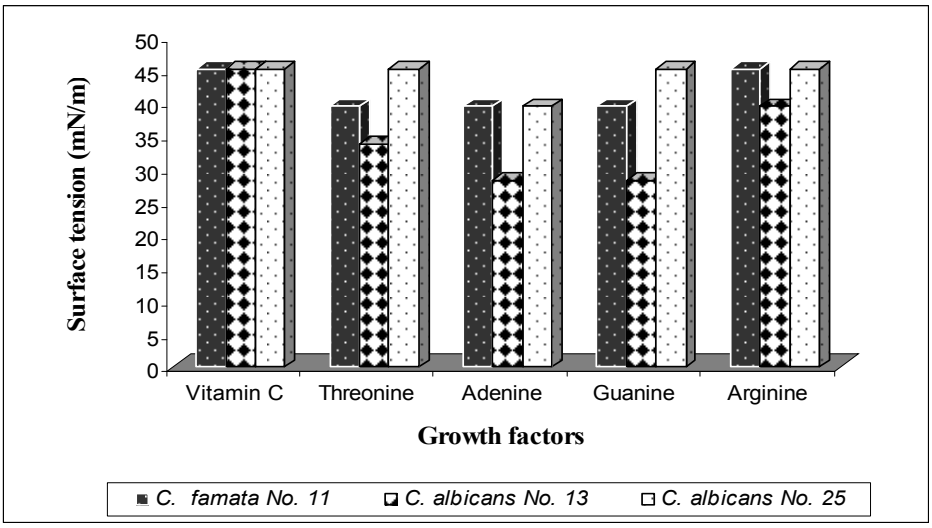

Fig. (14): Showing effect of different growth factors on biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 (Results are expressed as surface tension [mN/m]).

3.3 **Validation of optimum production of biosurfactant conditions:**

The best production of biosurfactant condition such as incubation period, pH, temperature, NaCl, sucrose concentration, $(NH_4)_2SO_4$ concentration and adenine for each organism was adjusted in the same culture to investigate the maximization production. The tested *Candida* strains were grown at pH 7 for 8 days. These *Candida* strains were incubated at 20ºC. A medium E was containing 5% NaCl, 1.0% sucrose, 12% $(NH_4)_2SO_4$ and adenine. Data in figure 15 revealed biosurfactant production with all strains examined and their oil displacement and surface tension.

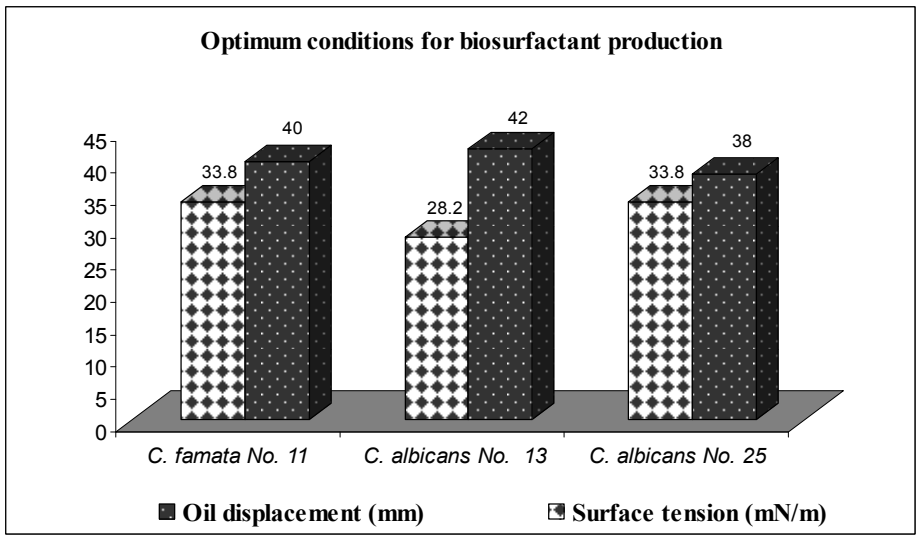

Fig. (15): Showing optimum condition for biosurfactant production by *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25.

4. DISCUSSION

Biosurfactants are a group of natural products of interest for biotechnological and industrial applications (**Desai and Banat, 1997; Bodour and Maier, 2002**). They have several advantages over their synthetic counterparts, such as lower toxicity, higher biodegradability (**Zajic *et al.*, 1977**), better environmental compatibility (**Georgiou *et al.*, 1992**), higher foaming (**Razafindralambo *et al.*, 1996**), high selectivity and specific activity at extreme temperature, pH and salinity (**Velikong and Kosaric, 1993; Ilori and Amund, 2001; Ilori *et al.*, 2005; Sarubbo *et al.*, 2006**), and the ability to be synthesized from renewable feedstock (**Desai and Banat, 1997**). For these indications the aim of this work was pointed out to optimization of culture condition for biosurfactant production from yeast in particular *Candida* strains.

Many of the potential applications that have been considered for biosurfactants depend on whether they can be produced economically; however, much effort in process optimization and at the engineering and biological levels have been carried out. Biosurfactant production from inexpensive waste substrates, which decreases their production cost (**Otto *et al.*, 1999 and Makkar and Cameotra, 2002**), has been reported. Therefore optimization of environmental conditions and nutritional requirements for *Candida famata* No. 11, *C. albicans* No.13 and *C. albicans* No. 25 was adapted to improve production conditions consequently enhancing biosurfactant production.

Environmental factors and growth conditions such as pH, temperature, agitation, and oxygen availability affect biosurfactant production through their effects on cellular growth or activity. The pH of the medium plays an important role in biosurfactant production by *Torulopsis bombicola* (**Gobbert *et al.*, 1984**). **Jagtap *et al.*, 2010** showed that maximum production of bioemulsifier was at pH 7 though less activity was at pHs 6, 8 and 9. Similarly, our data showed that pH 7 was the optimum one for production of biosurfactant. *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25 recorded 35, 42 and 32 mm oil displacement and 39.4, 28.2 and 39.4mN/m surface tension. Statistically, our result showed a highly significant correlation between pHs and each of oil displacement and surface tension ($r = 0.91, 0.99, 0.90, 0.90, 0.95$ and 0.95 respectively, $p < 0.05$ in all). Also, this observation was contrary to the Alasan produced by *A. radioresistent* KA53 which is reported to emulsification activity over a wide pH range 3.3-9.2 (**Navon-Venezia *et al.*, 1995**) and **Sudha *et al.*, 2010** observed that pH 3.5 was the optimum for the production of sophorolipid from *Candida tropicalis*.

Sudha *et al.*, 2010 observed that optimum incubation period was 7 days when compared with several cultural conditions for the maximum yield of sophorolipids. This observation is contrary to our study. On other hand our results showed that incubation period 8 days was a significant and optimum to produce biosurfactant by three tested *Candida* strains ($r = 0.97, 0.98, 0.99, 0.94, 0.99$ and 0.95 respectively, $P < 0.05$ in all). The maximum production of biosurfactant was indicated by results of oil displacement since they recorded 38, 41 and 36 mm for *Candida famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25, respectively.

Our data indicated that the optimum temperature for production of biosurfactant by all *Candida* isolates was mostly 20°C. *Candida famata* No. 11 and *C. albicans* No. 13 showed 28 and 30 mm oil displacement, and 33.8 and 28.2 mN/m surface tension, respectively. It observed also that oil displacement inversely proportional and surface tension directly proportional to temperatures. Also, our data showed that a highly significant correlation between incubation temperatures and each of oil displacement and surface tension ($r = 0.97, 0.99, 0.97, 0.90, 0.92$ and 0.96 respectively, $P < 0.05$ in all). In contrary **Sudha *et al.*, 2010** observed that temperature 30 ^{o}C was optimum for production of sophorolipid from *Candida tropicalis*. Also, **Jagtap *et al.*, 2010** found that optimum temperature for bioemulsifier production was 37 ^{o}C which can be attributed to the habitat of the organism. This observation is also in agreement with the report on bioemulsifier production by A. *junii* (Human skin isolate) which display temperature optima as 37 ^{o}C.

Abu-Ruwaida *et al.* (1991) reported that the salt concentration affected biosurfactant production depending on its effect on cellular activity. Some biosurfactant products, however, were not affected by salt concentrations up to 10% (w/v). Also, **Kiran *et al.*, 2010** found that the biosurfactant produced by the marine *Actinobacterium* was stable at high NaCl (up to 5% NaCl). Similarly our data revealed that production of biosurfactant was increased as NaCl concentration increased that reached maximum at 5% and then decreased down to 10% NaCl. However, **Ashish *et al.*, (2011)** showed that highest emulsification (50%) to biosurfactant produced by mutant strain of *Candida tropicalis* at salinity 6 %. Statistically, our data showed a highly significant correlation between NaCl concentrations and each of oil displacement and surface tension ($r = 0.76, 0.69, 0.87, 0.92, 0.64$ and 0.82 respectively, $P < 0.05$ in all).

Although there are a number of reports on the synthesis of biosurfactants by hydrocarbon-degrading microorganisms, some biosurfactants have been reported to be produced on water-soluble compounds

such as glucose, sucrose, glycerol, or ethanol (**Guerra-Santos *et al*., 1984; Cooper and Goldenberg, 1987; Palejwala and Desai, 1989; Passeri, 1992; Hommel, 1994**). Different concentrations of sucrose (as carbon source in medium E) were fulfillment the requirement of *Candida* species, but 1% (w/v) was optimum concentration. This means that the yeasts constitutively have all the enzymes involved in the biosynthesis of biosurfactant regardless of the carbon source supplied.

Medium E constituents other than carbon source also affect the production of biosurfactants. Among the inorganic salts tested, ammonium salt was preferred nitrogen source for biosurfactant production at concentration 12% (w/v). More likely, ammonium salts and urea were preferred by *Arthrobacter paraffineus* (**Duvnjak *et al*., 1983**), whereas nitrate supported maximum surfactant production in *P. aeruginosa* (**Guerra-Santos *et al*., 1984; Robert *et al*., 1989 and MacElwee *et al*., 1990**) and *Rhodococcus* spp. (**Abu-Ruwaida *et. al*., 1991**). **Okoro, (2011)** showed that media constituents can influence the emulsification activity of the bioemulsifier producing *Pseudomonas* isolates. He showed preference for $(NH_4)_2SO_4$ as opposed to $NaNO_3$ as the ideal source of nitrogen**.** This observation was in agreement with our findings. Our results showed a highly significant correlation between $(NH_4)_2SO_4$ concentrations and each of oil displacement and surface tension ($r = 0.94, 0.94, 0.99, 0.99, 0.98$ and 0.98 respectively, $P < 0.05$ in all). The optimum concentration of $(NH_4)_2SO_4$ was 12 % that recorded 33, 36 and 31 mm of oil displacement and 33.8, 28.2 and 39.4 mN/m of surface tension for *C. famata* No. 11, *C. albicans* No. 13 and *C. albicans* No. 25, respectively.

Many investigators have reported that the addition of biosurfactant precursors to the growth medium causes both qualitative and quantitative changes in the product. Similarly, increased production of biosurfactants containing different mono-, di, or trisaccharides was reported to occur in *Arthrobacter paraffineus* DSM 2567 (**Li *et al*., 1984**), *Corynebacterium* spp., *Nocardia* spp., and *Brevibacterium* spp. through supplementation with the corresponding sugar in the growth medium (**Brennan *et al*., 1970 and Itoh and Suzuki, 1974**). We modified this idea to investigate different precursors such as some amino acids, vitamin and nitrogenous bases for production of biosurfactant which resulted adenine as best precursor.

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