AJAB

Asian J Agric & Biol. 2023(4).
DOI: 10.35495/ajab.2022.146

Original Article

Optimization and antimicrobial properties of biosurfactant production by four indigenous soil bacterial species

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Received:
August 18, 2022
Accepted:
July 03, 2023
Published Online:
August 26, 2023

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Abstract

Biosurfactants of microbial origin are metabolites; hence their production is dependent on the growth of the producing microbe. The objective of this study was to assess the optimum conditions for biosurfactant by four bacterial species. Biosurfactant detection was carried out using emulsification index, drop collapse and oil displacement assays. Functional composition of the crude biosurfactant was determined using gas chromatography-mass spectroscopy (GC-MS). In the presence of *Pseudomonas fuscoginae* significantly higher EI_{24} of 53.98% and 52.60% was observed in media that contained glucose or sodium acetate as carbon source, respectively. When *P. fuscoginae* or *P. aeruginosa* was used for inoculation, highest EI_{24} of 61.18% and 48.40% was observed in media that contained potassium nitrate as nitrogen source. In the medium that was inoculated with either *B. subtilis* or *B. proteolyticus*, highest EI_{24} of 53.65% and 49.63% was observed in the presence of tryptone and peptone, respectively. At the respective pH used for investigation, significantly highest EI_{24} was observed at pH 6, when inoculated with the respective isolates. In the case of incubation temperature, positive results were obtained throughout the incubation period at 25 and 30 ^{o}C. This was also irrespective of the isolate used for inoculation. All the extracted biosurfactants showed antimicrobial potentials against the test pathogens used for investigation. Characterization of the crude biosurfactant revealed the presence of compounds with antimicrobial properties. The study was able to provide useful information on optimum conditions for biosurfactant production by test bacterial species and the potential for possible application of the biosurfactants as antimicrobial agents.

Keywords: Biosurfactant, Surface tension, Emulsification, Bacterial species

How to cite this:
Akpor OB, Ezekudo EO, Sobajo OA, Edoh PA and Mabayoje SO. Optimization and antimicrobial properties of biosurfactant production by four indigenous soil bacterial species. Asian J. Agric. Biol. 2023(4): 2022146. DOI: https://doi.org/10.35495/ajab.2022.146

Introduction

Surfactants are amphiphilic compounds (oil-soluble and water-soluble constituents) and are highly versatile. They have the ability to lower surface and interfacial tension between fluid phases, which could between liquid and liquid, gas and liquid or solid and liquid (Das et al., 2020). Because of their important

role in wetting, forming and breaking emulsions, formation and prevention of foaming, they are essential in many industries, such as in the detergent industry for production of soaps and detergents; the cosmetic industry for production of toothpastes, shampoos, conditioners, lotions, creams, shower gels, and several other self-care products (Cheng et al., 2020; Drakontis and Amin, 2020). They are also useful in pharmaceutical industries for drug production, and in the food industry for the production of salad creams, butter, mayonnaise and certain food additives. In addition, they are vital in the manufacture of waxes, dyes, paints and some agrochemicals such as insecticides (Gudiña et al., 2015; Santos et al., 2016).

Biosurfactants of microbial origin are metabolites; hence their production is dependent on the growth of the producing microbe (Santos et al., 2016; Sajid et al., 2020). The environmental factors that influence microbial growth are therefore important for biosurfactant production pathways. Variations in factors such as nature of carbon source, nitrogen source, nutrient accessibility and physicochemical conditions (temperature, salinity, pH, agitation speed and oxygen availability) influence the capacity of microorganisms to produce biosurfactants (Akbari et al., 2018). This study was therefore aimed at investigating the optimum conditions for biosurfactant production by four indigenous soil bacterial species and to assess the crude biosurfactants for antibacterial activity and potential of the biosurfactants produced.

Material and Methods

Preparation of bacterial isolates

Four bacterial species were used for the study. The bacteria were obtained from the Microbiology Unit of the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. The bacterial species were *Bacillus subtilis*, *Bacillus proteolyticus*, *Pseudomonas aeruginosa*, *Pseudomonas fuscoginae*. All were first streaked in nutrient agar plates to obtain distinct colonies. Distinct colonies were then streaked in stored as pure cultures on nutrient agar slants and stored in a refrigerator until when needed.

Screening for biosurfactant production

Biosurfactant detection was determined using emulsification index (EI_{24}), oil displacement and drop collapse tests. Emulsification index (EI_{24}) was determined as reported by Nwaguma et al. (2019) and calculated as:

$$E24(\%) = \frac{total\ height\ of\ emulsified\ layer}{total\ height\ of\ the\ liquid\ layer} X\ 100$$

Drop collapse and oil displacement tests were carried out as reported by Nayarisseri et al. (2018).

Optimization conditions for biosurfactant production

The effects of varying culture parameters (carbon source, nitrogen source, C/N ratio, temperature, pH, inoculum size and incubation time) on the biosurfactant-producing ability of the selected bacterial species were determined.

To study the effect of carbon source on biosurfactant production, to 250 mL-capacity conical flasks, 20 g/L of (as a nitrogen source) and 40 g/L of respective carbons sources (glucose, sucrose, lactose, maltose, fructose, mannitol, and sodium acetate, or 4 % v/v each of coconut oil, olive oil, and methanol) were weighed and added in 200 mL quantities. The media was homogenized and autoclaved at 121 °C for 15 min. After sterilization, 1 mL of an 18 h old broth culture of the respective isolates was inoculated and incubated at 37 °C. Every 24 h, for 120 h duration, the EI of the media were calculated, as described earlier.

For the effect of nitrogen source, the media composition was 40 g/L of the optimum carbon source, 20 g/L each of the respective nitrogen sources (peptone, tryptone, yeast extract, sodium nitrate, sodium nitrite, ascorbic acid, ammonium sulphate, and urea). The media was sterilized, inoculated with the respective isolate, incubated and EI calculated, as described earlier.

In the case of C/N ratio, concentrations of 40:8, 40:10, 40:20 and 40:30 g/L were used were for investigation, using the optimum carbon and nitrogen sources. The respective C:N proportions were added into respective flasks for preparation of the media. The media were sterilized, inoculated with the respective test isolates and EI estimated, as described earlier.

With respect to temperature variation, incubation temperatures of 25, 37 and 45 °C were used while pH used for investigation were 5, 7 and 9. Adjustment of pH of the media was carried out using 1 M HCl or 1 M NaOH, to obtain acidic and alkaline ranges, respectively. Media were composed of 40 g/L and 20

g/L of carbon and nitrogen sources, respectively. After sterilization and inoculation with the respective isolates, every 24 h, for 120-h duration, the EI of the media was calculated, as described earlier.

Experimental setups were carried out in 250 mL capacity conical flasks containing 200 mL of the composed medium.

Extraction, semi-purification and antimicrobial potential of crude biosurfactants

The acid precipitation method was used for extraction and semi-purification of the biosurfactants (Akpor et al., 2021). Following growth of the test bacterial species in liquid media, cell-free supernatants were obtained by centrifugation at 5000 rpm for 15 min. Each respective supernatant was transferred in 1 L-capacity beaker and pH adjusted to 2.0 with 1 M HCl and incubated at 4 ^{o}C for 24 h. After incubation, biosurfactant that was precipitated was extracted from the solution using chloroform and methanol (3:1 v/v) solvent mixture. Crude biosurfactant was obtained after evaporating the solvent at 60 ^{o}C in a water bath.

Antimicrobial potential of the biosurfactants

The bacterial isolates used were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*.

The agar well diffusion method was used in testing for antimicrobial potential of the extracted biosurfactants, as described by Akpor et al. (2021).

Results

Effect of carbon and nitrogen source

In the presence of *P. fuscoginae* significantly higher EI_{24} of 53.98% and 52.60% were observed in media that contained glucose or sodium acetate as carbon source, respectively. Similarly, when *B. subtilis* was used for inoculation, significantly higher EI_{24} of 47.12% and 43.45% were observed in media with glucose or sodium acetate, respectively. When *B. proteolyticus* or *P. aeruginosa* was used for inoculation no significant differences in EI_{24} were observed in media with the respective carbon sources (Fig. 1).

When *P. fuscoginae* or *P. aeruginosa* were used for inoculation, highest EI_{24} of 61.18% and 48.40% was observed in media that contained potassium nitrate as nitrogen source. In medium that was inoculated with either *B. subtilis* or *B. proteolyticus,* highest EI_{24} of 53.65% and 49.63% were observed in presence of tryptone and peptone, respectively. Generally, despite the differences in EI_{24} in medium with the respective nitrogen sources, these differences were not observed to be significant. This observation was also irrespective of the isolates used for inoculation (Fig. 1). In the case of carbon:nitrogen ratio (C:N), significantly higher EI_{24} was observed at 5:1 for media that were inoculated with *P. fuscoginae, B. proteolyticus* or *P. aeruginosa.* When inoculated with *B. subtilis,* although highest EI_{24} was observed in media with C:N of 5:1, this was not observed to be significant (Fig. 1).

The drop collapse and oil displacement tests revealed positive results throughout the 96 h incubation period in media that contained either glucose or acetate as carbon source. This observation was irrespective of the isolates that were used for inoculation. At the respective nitrogen sources, the drop collapse and oil displacement tests revealed positive results throughout the period of incubation in media that contained peptone or potassium nitrate (Table 1).

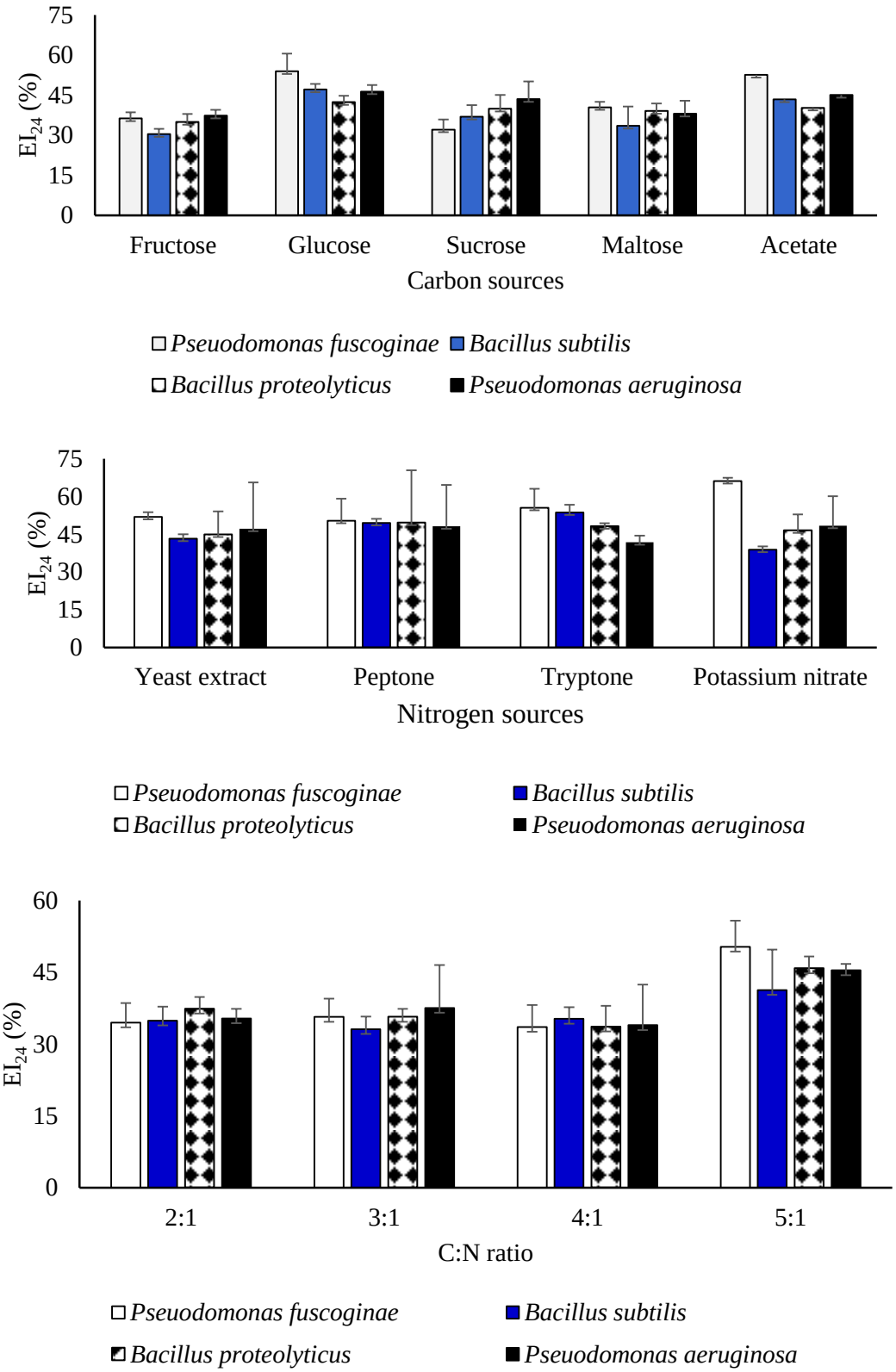

Figure-1. Effect of carbon and nitrogen sources on EI_{24} in presence of the isolates

Table-1. Effect of carbon source on biosurfactant detection using the drop collapse and oil displacement tests

Bacteria		Incubation period (h)							
		Drop collapse test				Oil displacement test			
	Carbon sources	24	48	72	96	24	48	72	96
Pseudomonas fuscoginae	Fructose	-	+	-	-	+	-	-	-
	Glucose	+	+	+	+	+	+	+	+
	Sucrose	-	-	-	-	-	-	-	-
	Maltose	-	-	-	-	-	-	-	-
	Acetate	+	+	+	+	+	+	+	+
Bacillus subtilis	Fructose	-	-	-	-	-	-	+	-
	Glucose	-	-	-	-	-	-	-	+
	Sucrose	-	-	-	-	-	-	-	-
	Maltose	-	-	-	-	-	-	-	-
	Acetate	+	+	-	-	-	-	-	+
Bacillus proteolyticus	Fructose	-	+	-	-	-	+	-	-
	Glucose	+	+	+	+	+	+	+	+
	Sucrose	-	-	-	-	-	-	-	-
	Maltose	-	-	-	-	-	-	-	+
	Acetate	+	+	-	-	+	+	+	+
Pseudomonas aeruginosa	Fructose	-	-	-	+	+	-	-	-
	Glucose	+	+	+	+	+	+	+	+
	Sucrose	-	-	-	-	-	-	-	+
	Maltose	-	-	-	-	-	-	-	-
	Acetate	+	+	+	+	+	+	+	+
	Nitrogen sources								
Pseudomonas fuscoginae	Yeast extract	-	-	-	-	-	-	-	-
	Peptone	+	+	+	+	+	+	+	+
	Tryptone	+	-	+	+	-	-	-	-
	Potassium nitrate	-	+	+	+	+	+	+	+
Bacillus subtilis	Yeast extract	-	-	-	-	-	+	-	-
	Peptone	+	+	+	+	+	+	+	+
	Tryptone	-	-	+	-	-	-	-	-
	Potassium nitrate	-	-	-	-	+	+	+	+
Bacillus proteolyticus	Yeast extract	-	-	-	-	-	-	-	-
	Peptone	+	+	+	+	-	-	-	-
	Tryptone	-	+	-	-	-	-	-	-
	Potassium nitrate	+	+	+	+	-	-	-	-
Pseudomonas aeruginosa	Yeast extract	-	-	-	-	-	-	-	-
	Peptone	+	+	+	+	+	+	+	+
	Tryptone	-	-	+	+	-	+	-	-
	Potassium nitrate	+	+	+	+	+	+	+	+

'-' and '+' represent negative and positive, respectively

Effect of pH and temperature

At the respective pH used for investigation, significantly highest EI_{24} was observed at pH 6, when inoculated with the respective isolates, with the exception of setup that was inoculated with *B. proteolyticus*, where highest EI_{24} was observed at pH 4. In the case of temperature, highest EI_{24} of 46.25%, 48.65%, 44.58% and 49.50% were observed at 25 ^{o}C in media that was inoculated with *P. fuscoginae*, *B. subtilis*, *B. proteolyticus*, and *P. aeruginosa*, respectively (Fig. 2).

The drop collapse and oil displacement tests showed positive results mainly in setups that were inoculated at pH 6 and 8. This observation was irrespective of the isolates used for inoculation and the duration of incubation. In the case of incubation temperature, positive results were obtained throughout the incubation period at 25 and 30 ^{o}C. This was also irrespective of the isolate used for inoculation (Table 2).

Antibacterial activity of the crude biosurfactants

The respective biosurfactants from the test bacterial species showed inhibition in growth of the pathogens used for investigation. Generally, concentration of 40 mg/L of the extracted biosurfactants did not show inhibition against the pathogens. A minimum inhibitory concentration (MIC) of 80 mg/L was established for the extracts against the test pathogens, except for the case of biosurfactant by *B. proteolyticus* that showed MIC of 120 mg/L against the test pathogens (Table 3).

Gas chromatograms of the extracted biosurfactants

The major compounds detected in the biosurfactant produced by the *P. fuscoginae* were Octadecenoic acid (Z)-, methyl ester (13.95%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (11.64%), Indoleacetic acid (11.04%), Dodecanoic acid (11.04), Heptadecane, 2-methyl- (10.34%), n-Hexadecanoic acid (9.74%), Benzene, 1,3-bis(1,1-dimethylethyl)- (7.46%) and Benzene, 1,3-bis(1,1-dimethylethyl) with peak area of 7.46% . In the case of the biosurfactant from the *P. aeruginosa*, the major compounds detected were 9-Octadecenoic acid (Z)-, methyl ester (14.43%), Heptadecane, 2-methyl- (12.34%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester(11.68%), n-Hexadecanoic acid (11.31%), Heptadecane, 2-methyl- (9.56%) and 7.79% Heptadecane, 2-methyl- (Table 4).

For the biosurfactant produced by the *B. subtilis*, the major compounds include 9-Octadecenoic acid (Z)-, methyl ester (13.59%), n-Hexadecanoic acid

(13.36%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (11.96%), Dodecanoic acid (9%), Dodecane, 2,6,10-trimethyl- (8.41%), Eicosanoic acid (8.08%) and peak area of 7.09% for Benzene, 1,3-bis(1,1-dimethylethyl)-. The major compounds detected in the biosurfactant produced by the *B. proteolyticus* were Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (11.88%), 9-Octadecenoic acid (Z)-, methyl ester (11.32%), Dodecanoic acid (10.22%), n-Hexadecanoic acid (9.94%), Eicosanoic acid (8.56%), Benzene, 1,3-bis(1,1-dimethylethyl)- (8.01%), Dodecane, 2,6,10-trimethyl- (8%) and Hexadecane,2,6,11,15 tetramethy (7.45%) (Table 5).

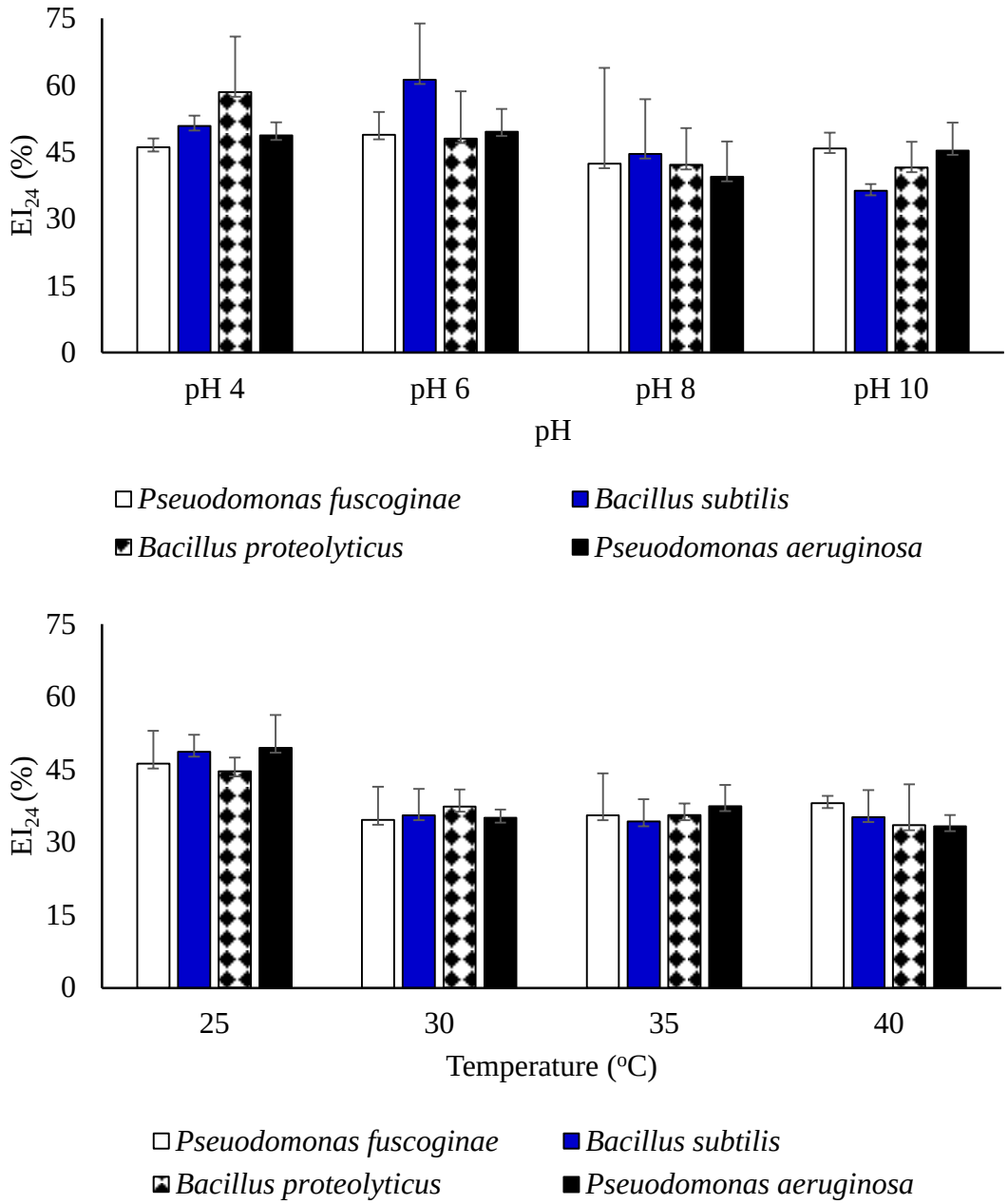

Figure-2. Effect of pH and temperature on EI_{24} in the presence of the isolates

Table-2. Effect of pH and temperature on biosurfactant detection using the drop collapse and oil displacement tests

Bacteria		Incubation period (h)							
		Drop collapse test				Oil displacement test			
	pH	24	48	72	96	24	48	72	96
Pseudomonas fuscoginae	4	-	-	-	+	-	-	-	-
	6	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+
	10	+	-	-	-	+	-	-	-
Bacillus subtilis	4	-	-	-	-	-	-	-	-
	6	-	-	-	-	+	+	+	-
	8	-	-	-	-	+	+	+	+
	10	-	-	-	-	-	-	-	-
Bacillus proteolyticus	4	+	-	-	-	+	+	-	-
	6	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	-
	10	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	4	+	-	-	+	+	+	-	-
	6	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+
	10	-	-	-	-	-	-	-	-
	Temperature (°C)								
Pseudomonas fuscoginae	25	+	+	+	+	+	+	+	+
	30	+	+	+	+	+	+	+	+
	35	-	-	-	+	-	-	-	+
	40	+	+	+	+	-	-	-	-
Bacillus subtilis	25	-	-	-	-	-	-	+	-
	30	-	-	-	-	+	+	-	-
	35	+	+	+	+	+	+	+	+
	40	-	-	-	-	-	-	-	-
Bacillus proteolyticus	25	+	+	+	+	+	+	+	+
	30	+	-	-	-	+	+	+	+
	35	-	-	-	-	+	+	+	+
	40	+	+	+	+	-	-	-	-
Pseudomonas aeruginosa	25	+	+	+	+	+	+	+	-
	30	-	-	-	-	+	+	+	-
	35	+	+	+	+	+	+	+	+
	40	-	-	+	-	-	-	+	+

'-' and '+' represent negative and positive, respectively

Table-3. Inhibitory potentials of the biosurfactants against selected pathogens

Concentration (mg/L)	Zones of inhibition (mm)				
	Xanthomonas campestris	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Escherichia coli*	*Salmonella typhi*
Pseudomonas fuscoginae					
40	-	-	-	-	-
80	15	16	17	17	17
120	19	16	15	15	15
160	20	21	19	23	17
200	18	20	20	22	19
Bacillus subtilis					
40	-	-	-	-	-
80	15	17	19	20	18
120	18	19	20	17	18
160	20	18	18	19	20
200	18	20	22	19	16
Bacillus proteolyticus					
40	-	-	-	-	-
80	-	-	-	-	-
120	19	16	18	18	17
160	17	21	17	18	19
200	19	17	16	21	19
Pseudomonas aeruginosa					
40	-	-	-	-	-
80	17	17	20	18	19
120	19	18	18	18	20
160	17	16	19	22	16
200	20	21	23	21	15

All values represent diameter of zone of inhibition in millimetres (mm)

Table-4. Detected compounds in the crude biosurfactant produced by the *Pseudomonas* species

Peak #	RT	Compound detected	Molecular formula	MW	Peak Area %	Comp. (%wt)
P. fuscoginae						
1	4.00	1-Undecanol	$C_{11}H_{24}O$	172	4.65	2.84
2	5.03	3-Dodecene (Z)-	$C_{12}H_{24}$	168	2.62	4.03
3	9.02	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190	7.46	5.05
4	9.80	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	134	1.15	5.51
5	12.49	Indoleacetic acid	$C_{10}H_8NO_2$	174	11.04	4.52
6	14.00	Dodecanoic acid	$C_{12}H_{24}O_2$	200	11.04	5.18
7	16.20	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	4.94	5.02
8	19.50	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	1.74	0.96
9	20.00	Heneicosane	$C_{21}H_{44}$	296	2.91	6.91
10	25.00	9,12-Octadecadienoic acid	$C_{14}H_{30}$	198	2.03	4.31
11	28.50	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	280	13.95	16.07
12	31.50	Hexadecane,2,6,11,15 tetramethy	$C_{20}H_{42}$	282	3.65	8.20
13	32.49	3-Eicosene, (E)-	$C_{20}H_{40}$	280	0.58	1.31
14	37.50	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.47	3.41
15	39.00	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	10.34	14.81
16	39.50	Eicosanoic acid	$C_{20}H_{40}O_2$	312	7.85`	4.06
17	40.18	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	11.63	6.95
P. aeruginosa						

1	4.00	1-Undecanol	$C_{11}H_{24}O$	172	4.47	2.81
2	5.03	3-Dodecene (Z)-	$C_{12}H_{24}$	168	2.75	3.14
3	9.02	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190	7.79	4.36
4	9.80	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	134	1.03	4.46
5	12.49	Indoleacetic acid	$C_{10}H_8NO_2$	174	0.69	3.47
6	14.00	Dodecanoic acid	$C_{12}H_{24}O_2$	200	5.15	5.29
7	16.20	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	2.06	5.64
8	19.50	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	3.09	1.73
9	20.00	Heneicosane	$C_{21}H_{44}$	296	2.41	7.04
10	25.00	9,12-Octadecadienoic acid	$C_{14}H_{30}$	198	4.81	4.82
11	28.50	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	280	14.43	16.57
12	31.50	Hexadecane,2,6,11,15 tetramethy	$C_{20}H_{42}$	282	5.81	8.51
13	32.49	3-Eicosene, (E)-	$C_{20}H_{40}$	280	0.34	1.04
14	37.50	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.31	3.31
15	39.00	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	12.34	14.75
16	39.50	Eicosanoic acid	$C_{20}H_{40}O_2$	312	9.56	4.00
17	40.18	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	11.68	6.97

Table-5. Detected compounds in the crude biosurfactant produced by the *Bacillus* species

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %wt
B. subtilis						
1	4.30	1-Undecanol	$C_{11}H_{24}O$	172	4.85	2.94
2	5.39	3-Dodecene (Z)-	$C_{12}H_{24}$	168	2.91	3.27
3	9.02	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190	7.09	4.37
4	9.98	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	134	1.29	4.80
5	12.49	Indoleacetic acid	$C_{10}H_8NO_2$	174	1.62	4.18
6	14.00	Dodecanoic acid	$C_{12}H_{24}O_2$	200	9.00	5.14
7	16.20	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	8.41	6.28
8	19.50	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	1.94	1.07
9	20.00	Heneicosane	$C_{21}H_{44}$	296	2.59	6.31
10	25.00	9,12-Octadecadienoic acid	$C_{14}H_{30}$	198	2.27	5.84
11	28.50	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	280	13.59	16.72
12	31.50	Hexadecane,2,6,11,15 tetramethy	$C_{20}H_{42}$	282	4.85	8.36
13	32.25	3-Eicosene, (E)-	$C_{20}H_{40}$	280	0.65	1.43
14	37.05	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.36	7.43
15	38.50	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	5.50	7.32
16	39.25	Eicosanoic acid	$C_{20}H_{40}O_2$	312	8.08	5.85
17	40.01	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	11.96	8.30
B. proteolyticus						
1	4.30	1-Undecanol	$C_{11}H_{24}O$	172	4.70	2.87
2	5.39	3-Dodecene (Z)-	$C_{12}H_{24}$	168	3.04	5.15
3	9.02	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190	8.01	5.90
4	9.98	Benzaldehyde, 2,4-dimethyl-	$C_9H_{10}O$	134	1.10	5.42
5	12.49	Indoleacetic acid	$C_{10}H_8NO_2$	174	1.38	6.75
6	14.00	Dodecanoic acid	$C_{12}H_{24}O_2$	200	10.22	7.00
7	16.20	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	8.00	8.63
8	19.50	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	1.66	0.93
9	20.00	Heneicosane	$C_{21}H_{44}$	296	3.31	7.13

10	25.00	9,12-Octadecadienoic acid	$C_{14}H_{30}$	198	1.93	3.15
11	28.50	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	280	11.32	14.80
12	31.50	Hexadecane,2,6,11,15 tetramethy	$C_{20}H_{42}$	282	7.45	9.53
13	32.25	3-Eicosene, (E)-	$C_{20}H_{40}$	280	0.65	1.63
14	37.05	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.94	3.00
15	38.50	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	6.80	6.28
16	39.25	Eicosanoic acid	$C_{20}H_{40}O_2$	312	8.56	4.53
17	40.01	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	11.88	7.20

Discussion

The present study showed glucose and sodium acetate to be better carbon sources for the optimum biosurfactant production by the bacterial species. It is reported that medium composition plays an important role on the type and concentration of biosurfactant produced by microorganisms (Silva et al., 2010). Earlier investigators reported that carbohydrates, vegetable oils, waste frying oils and hydrocarbons can be used as carbon sources for biosurfactant production (Deepika et al., 2016; Sena et al., 2018). Some studies have shown that using water-soluble carbon sources yield better biosurfactant than using hydrocarbons as sole carbon sources (Dastgheib et al., 2008; Abdel-Mawgoud et al., 2008; Haddad et al., 2009). A study by El-Sheshtawy and Doheim (2014), using *n*-hexadecane and glucose supplemented media reported glucose as the best carbon source for growth and biosurfactant production by *P. aeruginosa*. Also, glycerol and glucose has been reported to enhance biosurfactant production in the presence of *P. aeruginosa* (Joice and Parthasarathi, 2014).

This study revealed potassium nitrate as optimum nitrogen source for biosurfactant production by the isolates. In a related study by Sena et al. (2018), sodium nitrate was indicated to be unsuitable when they investigated the production of biosurfactants by soil fungi isolates. This observation negated the findings of Haddad et al. (2009) who reported urea and ammonium salts as ideal nitrogen sources in biosurfactant production. In a related study on the effect of several nitrogen sources (ammonium nitrate, ammonium phosphate, ammonium sulfate, ammonium chloride, peptone, potassium nitrate, yeast extract, and urea) on biosurfactant production in presence of *Bacillus subtilis* strain ANSKLAB03, Nayarisseri et al. (2018) reported highest emulsification index of 68 % and 60 % in media that contained yeast extract and urea, respectively. However, a similar study carried out by Nwaguma et al. (2019) reported that a combination of yeast extract and sodium nitrate was the more effective as nitrogen base for biosurfactant production.

In this study, remarkable EI was observed throughout the incubation time for all other C/N ratios. Increase in C/N ratio did not seem to have any significant effect on biosurfactant production which implies that lower C/N ratio is effective for biosurfactant production. This corroborates the findings of Elazzazy et al. (2015). A study on enhancement of *B. subtilis* lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration, showed that *B. subtilis* SPB1 had the highest biosurfactant production at a C/N ratio of 7:1, using ammonium chloride and urea as inorganic and organic nitrogen sources, respectively (Ghribi and Ellouze-Chaabouni, 2011). In another study, using glucose and NH_4NO_3 as carbon and nitrogen source, respectively, *Bacillus* sp. BMN14 was shown to achieve its highest decrease in surface tension (27mN/m) under a C/N ratio of 12.4 in comparison to ratios of 10.6 and 17.51 (Heryani and Putra, 2017). In most cases, it has been indicated that lower C/N ratios tend to favour biosurfactant production by *Bacillus* sp. (Nurfarahin et al., 2018).

A related study on the production of biosurfactant for microbial-enhanced oil recovery by bacteria isolated from oil contaminated wet soil showed a decrease in surface tension reduction with increment in C/N ratio from 10 to 20 (Agarwal and Sharma, 2009). Earlier investigators have reported maximum biosurfactant concentration and surface tension reduction when using a C/N ratio of 12.5 during biosurfactant production by *P. aeruginosa RS*29 (Saikia et al., 2012a). When using $(NH_4)_2SO_4$ and glycerol as nitrogen and carbon sources, respectively, Thavasi et al. (2011) observed highest reduction in surface tension at C/N ratio of 14 by *P. aeruginosa* UKMP14T. The study also proffered that C/N ratios greater than 20 retarded the growth of the

microorganism. Elazzazy et al. (2015) examined the effect of C/N ratios ranging from 10:1 to 70:1 on biosurfactant production and observed the lowest surface tension using a C/N ratio of 30:1.

From the findings of this study, optimum temperature for biosurfactant production was found to be 25 and 30 °C. When reporting on biosurfactant production by *B. subtilis* strain ANSKLAB03, Nayarisseri et al. (2018) stated that the optimum temperature for the bioprocess was observed to be 40 °C. In a similar study carried out with *P. aeruginosa* RS29, it was reported that maximum biosurfactant production by the bacterial species was attained at 37.5 °C (Saikia et al., 2012b). Similarly, a comparative study on biosurfactant production by *B. subtilis* and *P. aeruginosa* reported highest optical density at incubation temperature of 37 °C for both bacteria (Priya and Usharani, 2009).

In this study, pH 6 was observed as the optimum for biosurfactant production by all the bacterial species. In a similar study on *B. subtilis* N3-1P, maximum EI was attained at pH 6.41 (Moshtagh et al., 2019). In another study on *Bacillus cereus*, the maximum amount of biosurfactant was recovered after incubation at pH 6.5 (Basit et al., 2018). Previous studies have shown a neutral pH of 7.0 to be optimum for biosurfactant production by *P. aeruginosa* 181 and *P. aeruginosa* RS29 (Saikia et al., 2012b). In a study on the optimization of culture conditions for biosurfactant production from *P. aeruginosa* OCD1, Thavasi et al. (2011) observed that optimum pH for the bioprocess was 6.0.

The length of incubation has been shown to have substantial effect on production of biosurfactants as microorganisms are known to produce biosurfactants at varying time intervals. In this present study, 120 h incubation time was used for biosurfactant production. This corroborates the findings of Abdulsalam et al. (2016), which showed that biosurfactant concentration increased with longer incubation time.

This study revealed that the biosurfactant produced showed inhibition against all the tested bacterial isolates. A similar study on Rhamnolipids produced by *P. aeruginosa* stated that the biosurfactant had inhibitory potentials against *Staphylococcus aureus, S. epidermidis* and *Bacillus cereus* but insensitive to *E. coli* and *P. aeruginosa* (Bagheri et al., 2013). In another related study (Moryl et al., 2015), biosurfactants produced by *B. subtilis* on uropathogenic bacteria were indicated to have inhibitory potentials against the growth of strains *E. coli, K. pneumoniae, S. aureus* and *S. epidermidis* but not against strains of *P. aeruginosa*

Conclusion

From the study findings, glucose or sodium acetate and potassium nitrate were observed to be optimum carbon sources for biosurfactant production by the test bacterial species, respectively. A C:N of 5:1, pH range of 6-8 and temperature range of 25-30 ^{o}C were also observed to be optimum for biosurfactant production in presence of the test bacteria. All the crude biosurfactants extracted showed antibacterial activity against selected pathogens. Characterization of crude biosurfactants revealed the presence of several compounds of antimicrobial potentials. The study was able to provide useful information on optimum conditions for biosurfactant production by test bacterial species and the potential for possible application of the biosurfactants as antimicrobial agents.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

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Akpor OB: Conceptualized the study, approved the methodology, carried out experimental analysis, analyzed and interpreted the data carried literature review and contributed to the manuscript draft.
Ezekudo EO: Carried out literature search and experimental analysis, analyzed and interpreted the data and contributed to the manuscript draft.
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Edoh PA: Carried out experimental analysis and contributed to the manuscript draft.
Mabayoje SO: Carried out experimental analysis and contributed to the manuscript draft. All authors approved final draft of the manuscript.