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APPENDICES

APPENDIX A

Information and raw data in screening, isolation and identification

Table A-1 Growth of bacterial culture in mineral salt medium containing 2% wv^{-1} glucose as carbon source and without bacteria as control (Figure 4.1)

Time (hours)	Culture turbidity (OD_{600})				
	A102	A103	B202	P2	P3
0	0.045 ± 0.010	0.008 ± 0.001	0.007 ± 0.002	0.006 ± 0.002	0.023 ± 0.015
2	0.058 ± 0.003	0.017 ± 0.004	0.044 ± 0.029	0.015 ± 0.002	0.039 ± 0.006
3	0.083 ± 0.007	0.061 ± 0.007	0.478 ± 0.006	0.059 ± 0.001	0.406 ± 0.070
5	0.124 ± 0.011	0.076 ± 0.013	0.572 ± 0.019	0.068 ± 0.000	0.502 ± 0.056
6	0.236 ± 0.003	0.094 ± 0.020	0.702 ± 0.029	0.117 ± 0.002	0.689 ± 0.029
7	0.264 ± 0.031	0.171 ± 0.099	0.766 ± 0.009	0.273 ± 0.016	0.762 ± 0.005
8	0.631 ± 0.003	0.395 ± 0.194	0.907 ± 0.019	0.661 ± 0.024	1.005 ± 0.091
9	0.713 ± 0.023	0.722 ± 0.194	1.203 ± 0.221	1.002 ± 0.066	1.205 ± 0.027
10	0.939 ± 0.027	1.038 ± 0.179	1.311 ± 0.063	1.245 ± 0.060	1.302 ± 0.014
12	1.166 ± 0.055	1.279 ± 0.095	1.385 ± 0.062	1.323 ± 0.075	1.369 ± 0.018
14	1.407 ± 0.085	1.418 ± 0.108	1.572 ± 0.036	1.554 ± 0.130	1.599 ± 0.052
16	1.532 ± 0.092	1.613 ± 0.154	1.690 ± 0.038	1.771 ± 0.071	1.610 ± 0.072
24	1.723 ± 0.053	1.798 ± 0.093	1.874 ± 0.054	2.281 ± 0.005	1.819 ± 0.058
48	1.834 ± 0.103	1.842 ± 0.112	1.988 ± 0.115	2.287 ± 0.000	1.936 ± 0.061
72	1.965 ± 0.068	1.881 ± 0.074	2.018 ± 0.001	2.362 ± 0.006	2.350 ± 0.095
96	1.978 ± 0.071	1.886 ± 0.054	2.028 ± 0.004	2.363 ± 0.025	2.359 ± 0.101

A.1 The calculation of emulsification index (E_{24})

The emulsion stability was determined after 24 hours. The E_{24} was measured and calculated by measuring the emulsion layer thus formed as described by Cooper and Goldenberg (1987). The following equation was used to calculate % E_{24}

$$\text{Emulsification index (\% } E_{24}) = \frac{\text{Height of emulsion layer (cm.)}}{\text{Height of the oil plus emulsion layer (cm.)}} \times 100$$

For example

The height of emulsion layer = 0.95

The height of the oil plus emulsion layer = 1.25

Therefore, the emulsification index (% E_{24}) = $\frac{0.95}{1.25} \times 100$

Then, E_{24} = 76.0

APPENDIX B

Information and sequence fragment of 16S rDNA sequencing

The following formation is the sequence fragment of 16S rDNA sequencing of each bacterium with forward primer (63f) and reverse primer (1387r).

* 63f

TAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGA
GTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGA
AGCGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTG
CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCCAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGACGCAGCCATG
CCGCGTGTATGGAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATA
AGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGC
ACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTC
GAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATG
CGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCT
CAGGTGCGAAAGCGTGGAGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
CGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCG
ACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGATGCTACGCGAAGAACCTTACCTACTCTTGACAT
CCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATG
GCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAGGTCCCGCAACGAGCGCAACCCTTAT
CCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGG
AAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTAC
AATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTC
GTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGG
ATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGGCCGGCCTAACACAT
GCAAGTCCAGGCCTTGTACACTCCGGCCAGGCCTAACACATGCAAGTCCAGGCCTTGTAC
ACACCGGCCAGACCTAACACATGCAAGTCAGGCCTAACACATGCAAGTCCAGGCCTTGT
ACACTCCGGCC

1387r *

Figure B-1 A partial 16s rDNA sequence (1350 bp) of *Enterobacter* sp. P2

63f

CAGGCCTAACACATGCAAGTCGAACGGCAGCACGGGTGCTTGCACCTGGTGGCGAGTGG
CGAACGGGTGAGTAATACATCGGAACATGTCCTGTAGTGGGGGATAGCCCGGCGAAAGCC
GGATTAATACCGCATACGATCTACGGATGAAAGCGGGGGACCTTCGGGCCTCGCGCTATA
GGGTTGGCCGATGGCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCA
GTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCG
TGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATCCTTGGCTCTA
ATACAGTCGGGGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGGTGCAAGCGTTAAATCGGATTTACTGGGCGTAAAGCGTGCGCA
GGCGGTTTGCTAAGACCGATGTGAAATCCCCGGGGCTCAACCTGGGAACTGCATTGTGGA
CTGGCAGGCTAGAGTATGCCAGGGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTA
GAGATGTGGAGAATACCAATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGCTCATGCA
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCCTAAACGATGTC
AACTAGTTGTTGGGGATTCATTTCCTTAGTAACGTAGCTAACGCGTGAAGTTGACCGCCTG
GGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTG
GATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGGTCGGA
ATCCTGCTGAGAGGCGGGAGTGCTCGAAAGAGAACCGGCGCACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTA
GTTGCTACGCAAGAGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
TGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGAAC
AGAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCAGAAAACCGATCGTAGTCCGGATTG
CACTCTGCAACTCGAGTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGC
GGTGAATACGTTCCCGGGCCTTGTACACACCGGCC

1387r

Figure B-2 A partial 16s rDNA sequence (1354 bp) of *Burkholderia cepacia* P3

Table B-1 The blast N result of *Enterobacter* sp. P2 using 16S rDNA gene sequence comparison (http://www.ncbi.nlm.nih.gov/blast/)

Sequence identities (%)	Sequence accession number	Bacteria	References	Source of bacteria	Note
1) 99%	AJ853890	*Enterobacter hormaechei*	Hammond (2005)	Soil	-
2) 99%	EF138627	*Enterobacter* sp.	Sakai et al. (Unpublished)	Soil	-
3) 99%	AM184248	*Enterobacter* sp.	Abraham et al. (Unpublished)	River	-
4) 99%	DQ659161	*Enterobacter* sp.	Jia et al. (Unpublished)	Soil	biphenyl/polychlorinated
5) 99%	AJ853889	*Enterobacter cloacae*	Hammond (2005)	Soil	biphenyl degrader
6) 99%	AY995561	*Enterobacter hormaechei*	Gao (Unpublished)	Soil	-
7) 99%	Y17665	*Enterobacter cloacae*	Boye and Hansen (Unpublished)	Soil	-
8) 99%	AB114268	*Enterobacter* sp.		Soil	-
9) 99%	AM184238	*Enterobacter* sp.	Abraham et al. (Unpublished)	River	-
10) 99%	EF088367	*Enterobacter* sp.	Fanjat et al. (Unpublished)	Soil	-

Table B-2 The blast N result of *Burkholderia cepacia* P3 using 16S rDNA gene sequence comparison (http://www.ncbi.nlm.nih.gov/blast/)

Sequence identities (%)	Sequence accession number	Bacteria	References	Source of bacteria	Note
1) 99%	AB212239	*Burkholderia* sp.	Sakai et al. (Unpublished)	Soil	bacteria isolated from Japan
2) 99%	AB212230	*Burkholderia* sp.	Sakai et al. (Unpublished)	Soil	bacteria isolated from Japan
3) 99%	AB212227	*Burkholderia* sp.	Sakai et al. (Unpublished)	Soil	bacteria isolated from Japan
4) 99%	AF335494	*Burkholderia cepacia*	Kim et al. (2004)	Soil	a novel esterase gene
5) 99%	AY677089	*Burkholderia cepacia*	Ka (Unpublished)	Soil	-
6) 99%	AY769903	*Burkholderia* sp.	Ramette et al. (2005)	Soil	-
7) 99%	CP000459	*Burkholderia cenocepacia*	Copeland et al. (Unpublished)	Soil	-
8) 99%	AB252073	*Burkholderia cepacia*	Hashidoko et al. (Unpublished)	Soil	evolving N_2O from deforested tropical peatland
9) 99%	DQ847125	*Burkholderia* sp.	Shen et al. (Unpublished)	Soil	degradating ethylparaben
10) 99%	CP000379	*Burkholderia cenocepacia*	Copeland et al. (Unpublished)	Soil	-

APPENDIX C

D-glucose standard curve was used to determine the concentration of biosurfactant extracted from *Enterobacter* sp. P2 and *B. cepacia* P3 culture supernatant. The standard D-glucose was freshly prepared as a stock solution at 1.0 $mg.ml^{-1}$ by dilution to various concentrations at 10, 20, 30, 40 and 50 $\mu g.ml^{-1}$. The orcinol assay (Chandrasekaran *et al.*, 1980) (as descried in Method 3.4.3.2, Chapter 3) was used to directly assess the amount of glycolipids in the sample. The samples were analyzed by UV detector at a wavelength of 421 nm. The concentrations of glycolipids were calculated by comparing the data with the glucose standard curve (between 0 and 50 $\mu g.ml^{-1}$).

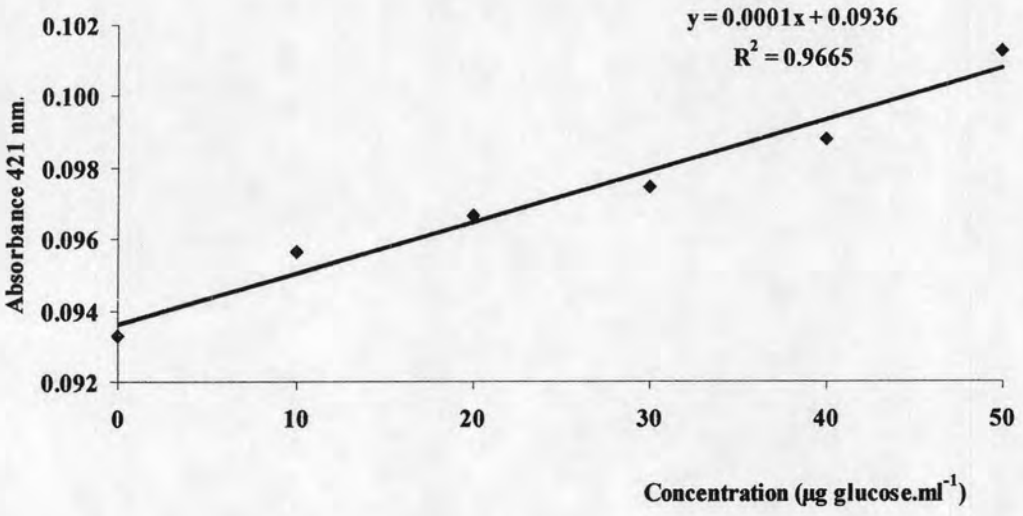

Figure C-1 Standard of D-glucose detect with orcinol method

Slope = 0.0001

The glycolipid concentration (μg) = (OD_{421}-0.0936)/0.0001

The culture supernatant (333 µl) was detected thus 1 ml had quantified the concentration of glucose

Example of calculation

The samples were prepared following in Method 3.4.3.1, Chapter 3 in order to determine with orcinol method.

Therefore, Absorbance 421 nm of sample	=	0.6762
Quantified the concentration of glucose	=	(OD_{421}-0.0936)/0.0001
	=	(0.6762-0.0936)/0.0001
	=	5,823 µg in 333 µl of sample
	=	17,486.49 $\mu g.ml^{-1}$
	=	17.49 $mg.ml^{-1}$
	=	17.49 $g.l^{-1}$

APPENDIX D

Identification of the biosurfactant type with Thin-layer chromatography (TLC)

Mobile phase: ethyl acetate: acetic acid: dH_2O = 6:3:2

Detection by 5% vv^{-1} H_2SO_4 in dH_2O

$$R_f = \frac{\text{Distance of sample (cm.)}}{\text{Distance of solvent (cm.)}}$$

The each isolates were treated following by the method 3.4.3.1, Chapter 3. Then, aqueous solution was kept warm (room temperature) and the sugar part was detected with thin-layer chromatography.

Table D-1 Analysis of the sugar part on biosurfactant of each isolated by thin-layer chromatography were demonstrated as retardation factor (n=3).

Concentration of samples	Distance of solvent (cm.)	Distance of samples (cm.)	R_f
1 mM glucose	7.9	3.7	0.47 ± 0.046
1 mM mannose	7.9	3.0	0.38 ± 0.046
1 mM rhamnose	7.9	5.1	0.65 ± 0.012
1.00 $\mu g.\mu l^{-1}$ *P. aeruginosa* A102	7.9	3.7	0.47 ± 0.056
1.20 $\mu g.\mu l^{-1}$ *P. aeruginosa* A103	7.9	3.7	0.47 ± 0.152
1.10 $\mu g.\mu l^{-1}$ *Pseudomonas* sp. B202	7.9	3.7	0.47 ± 0.029
1.21 $\mu g.\mu l^{-1}$ *Enterobacter* sp. P2	7.9	3.7	0.47 ± 0.180
1.30 $\mu g.\mu l^{-1}$ *B. cepacia* P3	7.9	3.7	0.47 ± 0.095

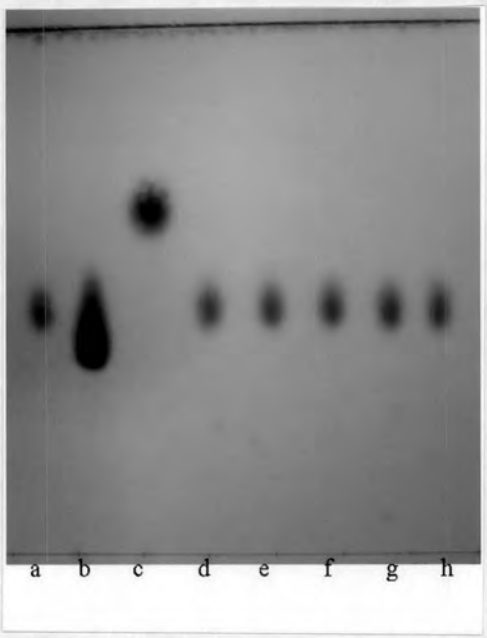

Figure D-1 Thin layer chromatography (TLC) analysis of the partially purified biosurfactant fraction. Lane (a) = 1 mM glucose; lane (b) = 1 mM mannose; lane (c) = 1 mM rhamnose; lane (d) = A102; lane (e) = A103; lane (f) = B202; lane (g) = partially purified biosurfactant of *Enterobacter* sp. P2 and lane (h) = *B. cepacia* P3. Each sample (2 µl) was developed with solvent system (by volume): ethyl acetate/acetic acid/water (6:3:2). For detection of components, the plate was sprayed with 5% vv^{-1} H_2SO_4 in dH_2O and heated at 110°C for 20 min.

APPENDIX E

Protein calibration curve

There are several methods to determined cell concentration such as wet cell weight, dry cell weight, viable count and etc., but they have the effect of technical error and their precision. Therefore, cell protein concentration is determined to represent cell the concentration for calculation of specific production rate in this test. Protein estimation is to determine the colorimetric assay of Lowry *et al.* (1951)

Prepare a standard curve of absorbance versus micrograms protein and determine amounts from the curve. Determine concentrations of original samples from the amount protein, volume/sample, and dilution factor, if any.

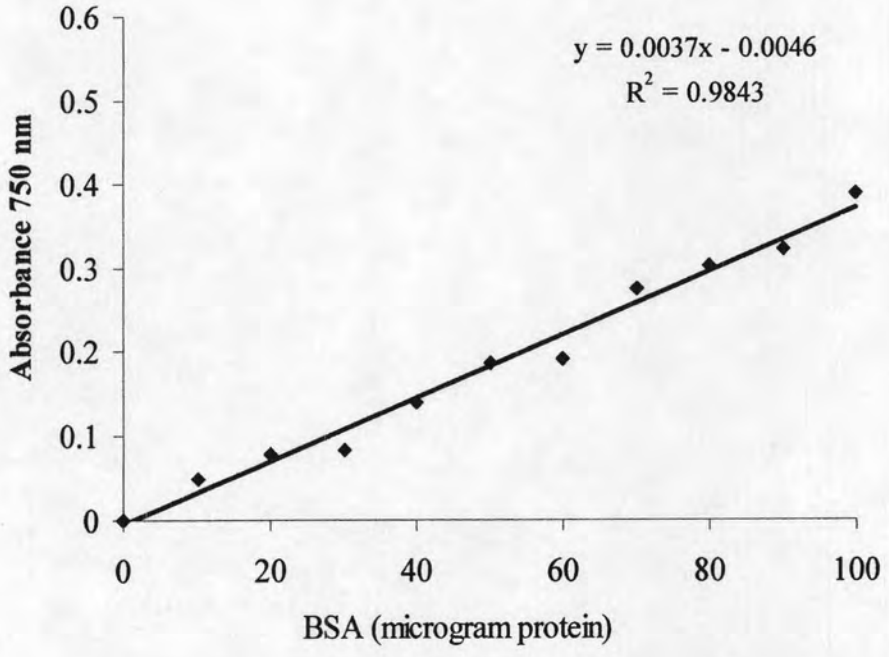

Figure E Standard curve of a modified Lowry method used to determine cell protein

Principle: (Dulley and Grieve (1975) and Lowry *et al.*, (1951))

Copper (color) ($CuSO_4.5H_2O$) + Protein —(Alkaline pH, Phenol reagent)→ Copper-protein complex (blue)

APPENDIX F

Information and raw data of physicochemical properties and activity of biosurfactant

F.1 Critical micelle concentration (CMC)

Critical micelle concentration was a measure of the concentration of a solution component which represents a critical value above which increasing concentration of that component forces the formation of micelles. CMC is determined by plotting the surface tension as a function of the logarithm of biosurfactant concentration and is found as the point at which the baseline of minimal surface tension intersects the slope where surface tension shows a linear decline.

Table F-1 Critical micelle concentration of glycolipid produced by *Enterobacter* sp. P2. Each point represented the mean and standard deviation of triplicate samples (Figure 4.18).

Concentration ($mg.l^{-1}$)	Log conc. ($mg.l^{-1}$)	Critical micelle concentration (CMC)
0.00	0.00	71.83 ± 0.258
1.26	0.10	50.83 ± 0.258
1.58	0.20	45.67 ± 0.516
2.00	0.30	36.43 ± 0.361
2.50	0.40	34.77 ± 0.225
3.16	0.50	31.27 ± 0.723
10.00	1.00	28.73 ± 0.225
31.62	1.50	27.47 ± 0.052
50.00	1.70	24.33 ± 0.258
100.00	2.00	25.20 ± 0.237
316.23	2.50	24.37 ± 0.137
582.00	2.76	24.20 ± 0.155
1,000.00	3.00	24.27 ± 0.052
3,162.28	3.50	24.23 ± 0.207

Table F-2 Critical micelle concentration of glycolipid produced by *B. cepacia* P3. Each point represented the mean and standard deviation of triplicate samples (Figure 4.18).

Concentration (mg.l^{-1})	**Log conc. (mg.l^{-1})**	**Critical micelle concentration (CMC)**
0.0	0.0	71.83 ± 0.258
3.2	0.5	46.17 ± 0.258
10.0	1.0	44.67 ± 0.258
31.6	1.5	40.37 ± 0.493
100.0	2.0	36.10 ± 0.155
316.2	2.5	35.17 ± 0.186
1,000.0	3.0	34.20 ± 0.237
1,258.9	3.1	30.03 ± 0.052
1,584.9	3.2	30.70 ± 1.008
1,995.3	3.3	30.53 ± 0.750
2,511.9	3.4	30.37 ± 0.493
3,162.3	3.5	30.03 ± 0.052
12,323.0	4.1	30.03 ± 0.052

Example of calculation

From figure 4.18 in Chapter 4:

The intercept of two straight lines from the concentration-dependent and concentration-independent sections

For example:

Concentration of *Enterobacter* sp. P2 = Log 0.52 mg.l^{-1}

Therefore, the concentration of *Enterobacter* sp. P2 = 3.31 mg.l^{-1}

Critical micelle concentration = 3.31 mg.l^{-1}

F.2 Stability of biosurfactant at various temperatures of 30–75°C and pH

Table F-3 Stability of the emulsification index (E_{24}) at 30°C, 37°C and 45°C of *Enterobacter* sp. P2 and *B. cepacia* P3 for at least three months (n=3) (Figure 4.19-4.21).

Day (s)	E_{24} of *Enterobacter* sp. P2			E_{24} of *Burkholderia cepacia* P3		
	30°C	37°C	45°C	30°C	37°C	45°C
1	83.77 ± 2.25	88.88 ± 0.77	83.66 ± 0.82	86.19 ± 0.55	88.71 ± 0.58	87.31 ± 3.11
3	83.77 ± 2.25	88.88 ± 0.77	79.29 ± 5.44	86.19 ± 0.55	88.71 ± 0.58	76.13 ± 2.82
6	83.77 ± 2.25	88.88 ± 0.77	76.79 ± 2.06	86.19 ± 0.55	88.71 ± 0.58	71.05 ± 3.04
7	83.77 ± 2.25	85.88 ± 1.88	70.00 ± 0.00	86.19 ± 0.55	86.19 ± 0.55	65.48 ± 1.37
14	83.77 ± 2.25	85.88 ± 1.88	65.85 ± 5.47	86.19 ± 0.55	86.19 ± 0.55	52.53 ± 6.32
21	82.99 ± 3.14	85.88 ± 1.88	54.41 ± 5.09	86.19 ± 0.55	86.19 ± 0.55	44.87 ± 2.53
28	83.77 ± 2.25	85.88 ± 1.88	42.28 ± 5.52	86.19 ± 0.55	86.19 ± 0.55	33.41 ± 0.09
35	78.56 ± 0.65	85.88 ± 1.88	36.72 ± 3.62	86.19 ± 0.55	86.19 ± 0.55	22.59 ± 2.37
42	78.56 ± 0.65	85.88 ± 1.88	15.16 ± 1.71	75.74 ± 0.85	86.19 ± 0.55	12.44 ± 0.05
49	72.44 ± 6.43	85.88 ± 1.88	0.00 ± 0.00	75.74 ± 0.85	82.14 ± 4.12	0.00 ± 0.00
56	66.25 ± 5.02	85.88 ± 1.88	0.00 ± 0.00	75.74 ± 0.85	78.57 ± 0.00	0.00 ± 0.00
63	66.25 ± 5.02	83.48 ± 2.57	0.00 ± 0.00	75.74 ± 0.85	78.57 ± 0.00	0.00 ± 0.00
85	66.25 ± 5.02	78.86 ± 2.76	0.00 ± 0.00	66.56 ± 2.76	78.57 ± 0.00	0.00 ± 0.00
115	61.01 ± 3.89	67.57 ± 1.04	0.00 ± 0.00	66.56 ± 2.76	71.54 ± 0.79	0.00 ± 0.00
145	61.01 ± 3.89	56.47 ± 3.07	0.00 ± 0.00	66.56 ± 2.76	63.44 ± 0.99	0.00 ± 0.00

Table F-4 Stability of the emulsification index (E_{24}) at various of pH 2-12 of *Enterobacter* sp. P2 and *B. cepacia* P3 for one month (n = 3) (Figure 4.22-4.23).

Day	E_{24} of *Enterobacter* sp. P2 at pH							E_{24} of *Burkholderia cepacia* P3 at pH						
(s)	2	4	6	7	9	10	12	2	4	6	7	9	10	12
1	40.00 ± 3.641	61.03 ± 0.794	71.79 ± 1.986	94.87 ± 1.986	64.62 ± 2.481	48.21 ± 1.731	35.13 ± 3.531	32.05 ± 7.161	55.90 ± 2.781	71.28 ± 4.031	92.31 ± 3.440	64.10 ± 1.986	70.18 ± 6.880	33.59 ± 2.212
3	36.92 ± 2.350	55.23 ± 4.280	71.79 ± 1.986	94.87 ± 1.986	64.54 ± 2.447	48.21 ± 1.731	35.13 ± 3.531	31.59 ± 0.000	53.47 ± 5.658	67.86 ± 3.491	92.31 ± 3.440	52.30 ± 5.658	67.57 ± 1.407	8.23 ± 2.070
6	35.21 ± 1.189	47.55 ± 3.984	71.79 ± 1.986	94.87 ± 1.986	54.62 ± 0.469	44.56 ± 6.350	34.50 ± 2.887	30.57 ± 0.984	50.48 ± 1.374	58.33 ± 9.623	87.80 ± 4.861	48.36 ± 0.000	54.74 ± 1.532	0.00 ± 0.000
9	25.08 ± 1.986	22.58 ± 6.258	65.82 ± 2.940	94.87 ± 1.986	46.92 ± 1.340	35.09 ± 2.026	29.21 ± 12.459	27.11 ± 2.589	45.56 ± 5.133	58.34 ± 9.624	87.79 ± 2.892	45.53 ± 5.774	38.33 ± 1.723	0.00 ± 0.000
12	11.77 ± 1.057	11.50 ± 4.041	51.53 ± 1.316	94.87 ± 1.986	46.92 ± 1.340	35.09 ± 2.026	20.18 ± 4.047	25.74 ± 0.657	41.84 ± 9.422	48.11 ± 6.028	88.55 ± 2.507	33.33 ± 5.190	27.88 ± 1.166	0.00 ± 0.000
15	8.36 ± 1.155	10.30 ± 2.379	35.09 ± 2.026	94.87 ± 1.986	38.59 ± 2.558	34.40 ± 1.443	15.56 ± 3.204	16.25 ± 3.886	38.53 ± 13.244	32.59 ± 0.000	88.55 ± 2.507	33.33 ± 0.269	27.50 ± 2.887	0.00 ± 0.000
18	8.36 ± 1.155	4.18 ± 4.827	22.60 ± 2.032	90.36 ± 2.530	32.01 ± 1.524	29.14 ± 3.316	15.56 ± 3.204	16.25 ± 3.886	31.05 ± 3.037	30.57 ± 1.270	88.55 ± 2.507	33.33 ± 5.027	19.21 ± 2.026	0.00 ± 0.000
21	0.00 ± 0.000	0.00 ± 0.000	22.32 ± 5.854	90.36 ± 2.530	22.32 ± 5.854	21.57 ± 5.658	15.56 ± 3.204	16.25 ± 3.886	22.28 ± 5.519	27.11 ± 3.343	74.08 ± 2.166	32.00 ± 3.027	19.21 ± 0.031	0.00 ± 0.000
24	0.00 ± 0.000	0.00 ± 0.000	8.05 ± 1.485	88.54 ± 5.230	8.05 ± 1.485	14.74 ± 0.000	7.11 ± 2.590	10.00 ± 11.547	14.71 ± 6.795	27.11 ± 3.343	74.08 ± 2.166	13.05 ± 1.450	18.56 ± 0.003	0.00 ± 0.000
27	0.00 ± 0.000	0.00 ± 0.000	8.05 ± 1.485	88.54 ± 5.230	0.00 ± 0.000	8.33 ± 5.190	0.00 ± 0.000	0.00 ± 0.000	10.00 ± 11.547	15.74 ± 0.849	63.39 ± 9.942	13.05 ± 4.047	15.09 ± 0.023	0.00 ± 0.000
30	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	88.54 ± 5.230	0.00 ± 0.000	7.88 ± 1.166	0.00 ± 0.000	0.00 ± 0.000	6.98 ± 2.281	6.25 ± 5.017	52.76 ± 0.958	6.70 ± 3.204	8.27 ± 0.027	0.00 ± 0.000

F-3 Effect of biosurfactant-producing bacteria in Luria Bertani broth (LB) and nutrient broth (NB)

Table F-5 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 grown in mineral salt medium (MSM) containing 2% wv^{-1} glucose as carbon source: the control as MSM, LB and NB. Each point represented the mean and standard deviation of triplicate samples (Figure 4.24).

Time (hours)	OD_{600}, *Enterobacter* sp. P2			OD_{600}, *Burkholderia cepacia* P3		
	MSM	LB	NB	MSM	LB	NB
0	0.006 ± 0.002	0.138 ± 0.015	0.138 ± 0.017	0.023 ± 0.015	0.074 ± 0.013	0.126 ± 0.002
2	0.015 ± 0.002	0.164 ± 0.012	0.234 ± 0.004	0.039 ± 0.006	0.131 ± 0.008	0.215 ± 0.018
5	0.068 ± 0.000	0.158 ± 0.030	0.369 ± 0.046	0.502 ± 0.056	0.184 ± 0.010	0.288 ± 0.017
7	0.273 ± 0.016	0.225 ± 0.011	0.453 ± 0.043	0.762 ± 0.005	0.261 ± 0.008	0.376 ± 0.031
8	0.661 ± 0.024	0.374 ± 0.007	0.558 ± 0.007	1.005 ± 0.091	0.423 ± 0.011	0.459 ± 0.027
10	1.245 ± 0.060	0.654 ± 0.005	1.126 ± 0.020	1.302 ± 0.014	0.646 ± 0.018	0.838 ± 0.032
12	1.323 ± 0.075	0.880 ± 0.050	1.534 ± 0.036	1.369 ± 0.018	1.015 ± 0.017	1.056 ± 0.017
14	1.554 ± 0.130	1.070 ± 0.035	1.591 ± 0.017	1.599 ± 0.052	1.258 ± 0.018	1.268 ± 0.021
16	1.771 ± 0.071	1.458 ± 0.037	1.681 ± 0.009	1.610 ± 0.072	1.647 ± 0.012	1.497 ± 0.002
24	2.281 ± 0.005	1.969 ± 0.021	1.761 ± 0.044	1.819 ± 0.058	1.825 ± 0.025	1.652 ± 0.006
48	2.287 ± 0.000	2.034 ± 0.027	1.840 ± 0.022	1.936 ± 0.061	1.972 ± 0.018	1.753 ± 0.039
72	2.362 ± 0.006	2.158 ± 0.076	1.960 ± 0.008	2.350 ± 0.095	2.029 ± 0.018	1.850 ± 0.026
96	2.363 ± 0.025	2.142 ± 0.053	1.959 ± 0.006	2.359 ± 0.101	2.030 ± 0.097	1.851 ± 0.016

Table F-6 Glycolipid production of *Enterobacter* sp. P2 and *B. cepacia* P3 when cultivated in mineral salt medium (MSM) containing 2% wv^{-1} glucose as carbon source, Luria Bertani broth (LB) and nutrient broth (NB). Each point represented the mean and standard deviation of triplicate samples (Figure 4.25).

Productive condition	**Glycolipid concentration ($g.l^{-1}$)**	
(Medium)	***Enterobacter* sp. P2**	***B. cepacia* P3**
Mineral salt medium (MSM)	17.49 ± 0.338	37.01 ± 0.118
Luria Bertani broth (LB)	12.44 ± 0.513	26.85 ± 0.306
Nutrient broth (NB)	13.95 ± 0.610	27.14 ± 1.217

APPENDIX G

Information and raw data of biosurfactant-producing bacteria by two newly bacteria isolate (*Enterobacter* sp. P2 and *Burkholderia cepacia* P3)

G.1 Biosurfactant-producing bacteria information

In this work, three carbon sources which various concentrations e.g. 11.1 mM (2% wv^{-1}), 44.4 mM (8% wv^{-1}) and 83.3 mM (15% wv^{-1}), including glucose, maltose and sucrose were examined for their effectiveness on glycolipid production.

Table G-1 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 in mineral salt medium containing 2% wv^{-1} (11.1 mM) carbon source at 37°C, 250 rpm (n = 3) (Figure 4.26).

Time	OD_{600}, *Enterobacter* sp. P2			OD_{600}, *Burkholderia cepacia* P3		
(hours)	Glucose	Maltose	Sucrose	Glucose	Maltose	Sucrose
0	0.006 ± 0.002	0.007 ± 0.003	0.002 ± 0.001	0.023 ± 0.015	0.025 ± 0.020	0.011 ± 0.005
2	0.015 ± 0.002	0.017 ± 0.007	0.020 ± 0.021	0.039 ± 0.006	0.158 ± 0.018	0.076 ± 0.035
5	0.068 ± 0.000	0.036 ± 0.016	0.037 ± 0.016	0.502 ± 0.056	0.238 ± 0.001	0.258 ± 0.125
7	0.273 ± 0.016	0.089 ± 0.012	0.071 ± 0.021	0.762 ± 0.005	0.274 ± 0.019	0.412 ± 0.188
8	0.661 ± 0.024	0.312 ± 0.056	0.141 ± 0.026	1.005 ± 0.091	1.295 ± 0.047	0.573 ± 0.165
10	1.245 ± 0.060	0.476 ± 0.105	0.350 ± 0.036	1.302 ± 0.014	1.379 ± 0.069	0.667 ± 0.179
12	1.323 ± 0.075	0.809 ± 0.011	0.530 ± 0.027	1.369 ± 0.018	1.413 ± 0.072	0.970 ± 0.136
14	1.554 ± 0.130	1.043 ± 0.048	0.799 ± 0.029	1.599 ± 0.052	1.456 ± 0.077	1.151 ± 0.010
16	1.771 ± 0.071	1.071 ± 0.060	1.030 ± 0.031	1.610 ± 0.072	1.497 ± 0.093	1.293 ± 0.104
24	2.281 ± 0.005	1.181 ± 0.073	1.126 ± 0.039	1.819 ± 0.058	1.538 ± 0.123	1.597 ± 0.039
48	2.287 ± 0.000	1.422 ± 0.057	1.210 ± 0.084	1.936 ± 0.061	1.597 ± 0.118	1.727 ± 0.109
72	2.362 ± 0.006	1.607 ± 0.027	1.249 ± 0.042	2.350 ± 0.095	1.807 ± 0.033	1.816 ± 0.029
96	2.363 ± 0.025	1.603 ± 0.026	1.247 ± 0.045	2.359 ± 0.101	1.802 ± 0.033	1.784 ± 0.069

Table G-2 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 in mineral salt medium containing 8% wv^{-1} (44.4 mM) carbon source at 37°C, 250 rpm (n = 3) (Figure 4.27).

Time	OD_{600}, *Enterobacter* sp. P2			OD_{600}, *Burkholderia cepacia* P3		
(hours)	Glucose	Maltose	Sucrose	Glucose	Maltose	Sucrose
0	0.189 ± 0.001	0.003 ± 0.001	0.083 ± 0.020	0.059 ± 0.052	0.121 ± 0.057	0.074 ± 0.061
2	0.247 ± 0.015	0.012 ± 0.004	0.084 ± 0.026	0.176 ± 0.023	0.173 ± 0.037	0.130 ± 0.026
5	1.182 ± 0.046	0.207 ± 0.076	0.102 ± 0.002	0.261 ± 0.006	0.223 ± 0.010	0.168 ± 0.057
7	1.608 ± 0.019	0.311 ± 0.017	0.116 ± 0.012	0.605 ± 0.048	0.470 ± 0.014	0.247 ± 0.020
8	1.720 ± 0.028	0.504 ± 0.056	0.110 ± 0.027	1.361 ± 0.033	1.101 ± 0.074	0.311 ± 0.025
10	1.818 ± 0.023	0.560 ± 0.030	0.127 ± 0.029	1.756 ± 0.022	1.302 ± 0.070	0.351 ± 0.020
12	1.931 ± 0.019	0.614 ± 0.046	0.153 ± 0.040	1.988 ± 0.066	1.503 ± 0.082	0.373 ± 0.027
14	2.051 ± 0.027	0.662 ± 0.035	0.193 ± 0.066	2.070 ± 0.098	1.570 ± 0.114	0.392 ± 0.024
16	2.103 ± 0.003	0.678 ± 0.049	0.220 ± 0.075	2.110 ± 0.091	1.606 ± 0.154	0.427 ± 0.041
24	2.169 ± 0.014	0.769 ± 0.032	0.275 ± 0.111	2.155 ± 0.075	1.692 ± 0.079	0.454 ± 0.034
48	2.332 ± 0.025	0.847 ± 0.021	0.332 ± 0.054	2.257 ± 0.045	1.811 ± 0.066	0.503 ± 0.022
72	2.374 ± 0.014	0.993 ± 0.005	0.659 ± 0.018	2.341 ± 0.021	1.852 ± 0.048	0.530 ± 0.032
96	2.372 ± 0.014	0.984 ± 0.001	0.653 ± 0.024	2.335 ± 0.012	1.821 ± 0.037	0.589 ± 0.047

Table G-3 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 in mineral salt medium containing 15% wv^{-1} (83.3 mM) carbon source at 37°C, 250 rpm (n = 3) (Figure 4.28).

Time	OD_{600}, *Enterobacter* sp. P2			OD_{600}, *Burkholderia cepacia* P3		
(hours)	Glucose	Maltose	Sucrose	Glucose	Maltose	Sucrose
0	0.024 ± 0.015	0.037 ± 0.006	0.035 ± 0.006	0.042 ± 0.006	0.019 ± 0.021	0.038 ± 0.013
2	0.187 ± 0.056	0.050 ± 0.012	0.062 ± 0.008	0.067 ± 0.013	0.141 ± 0.033	0.112 ± 0.015
5	0.346 ± 0.152	0.089 ± 0.001	0.085 ± 0.011	0.143 ± 0.026	0.198 ± 0.008	0.175 ± 0.018
7	0.729 ± 0.123	0.167 ± 0.059	0.110 ± 0.010	0.238 ± 0.064	0.268 ± 0.024	0.264 ± 0.007
8	0.941 ± 0.180	0.246 ± 0.017	0.152 ± 0.031	0.347 ± 0.024	0.348 ± 0.022	0.281 ± 0.004
10	1.180 ± 0.195	0.248 ± 0.016	0.173 ± 0.043	0.516 ± 0.022	0.406 ± 0.003	0.294 ± 0.002
12	1.408 ± 0.198	0.618 ± 0.020	0.207 ± 0.042	0.802 ± 0.021	0.499 ± 0.028	0.308 ± 0.006
14	1.636 ± 0.185	0.682 ± 0.039	0.263 ± 0.032	0.902 ± 0.002	0.681 ± 0.101	0.325 ± 0.003
16	1.759 ± 0.186	0.692 ± 0.042	0.349 ± 0.032	1.098 ± 0.067	1.023 ± 0.111	0.347 ± 0.001
24	1.861 ± 0.112	0.706 ± 0.032	0.390 ± 0.027	1.425 ± 0.048	1.152 ± 0.040	0.360 ± 0.006
48	1.935 ± 0.078	0.730 ± 0.020	0.450 ± 0.040	1.782 ± 0.081	1.343 ± 0.059	0.380 ± 0.010
72	1.985 ± 0.017	0.755 ± 0.003	0.539 ± 0.011	2.053 ± 0.014	1.605 ± 0.006	0.406 ± 0.004
96	1.989 ± 0.010	0.751 ± 0.002	0.536 ± 0.010	2.019 ± 0.032	1.594 ± 0.005	0.402 ± 0.003

Table G-4 Biosurfactant production of the two bacterial isolates in mineral salt medium containing varies type and concentration of carbon source at 37°C, 250 rpm (n = 3) (Figure 4.29).

Productive condition	**Glycolipid concentration ($g.l^{-1}$)**	
	***Enterobacter* sp. P2**	***B. cepacia* P3**
Culture medium	17.49 ± 0.338	37.0149 ± 0.118
11.1 mM Glucose	17.4949 ± 0.338	37.0149 ± 0.118
11.1 mM Maltose	5.8349 ± 0.030	13.9649 ± 0.819
11.1 mM Sucrose	3.0749 ± 0.044	3.7149 ± 0.006
44.4 mM Glucose	14.2749 ± 0.111	28.4049 ± 0.770
44.4 mM Maltose	10.7749 ± 0.147	9.7549 ± 0.075
44.4 mM Sucrose	9.7749 ± 0.002	3.1049 ± 0.020
83.3 mM Glucose	3.9749 ± 0.096	16.6449 ± 0.821
83.3 mM Maltose	2.8549 ± 0.017	3.1649 ± 0.110
83.3 mM Sucrose	0.5049 ± 0.021	1.6449 ± 0.076

In nitrogen-free medium, the least reduction in production was achieved, whereas sodium nitrate, ammonium sulphate and urea were the best sources of nitrogen of those tested.

Table G-5 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 in mineral salt medium containing 2% wv^{-1} (11.1 mM) carbon source and $NaNO_3$ as nitrogen source at 37°C, 250 rpm (n = 3) (Figure 4.30).

Time	OD_{600}, *Enterobacter* sp. P2, 11.1 mM Glucose + $NaNO_3$				OD_{600}, *Burkholderia cepacia* P3, 11.1 mM Glucose + $NaNO_3$			
(hours)	Control	15 mM	75 mM	150 mM	Control	15 mM	75 mM	150 mM
0	0.006 ± 0.002	0.193 ± 0.001	0.195 ± 0.000	0.165 ± 0.030	0.023 ± 0.015	0.195 ± 0.001	0.196 ± 0.002	0.196 ± 0.002
2	0.015 ± 0.002	0.237 ± 0.002	0.261 ± 0.004	0.193 ± 0.005	0.039 ± 0.006	0.240 ± 0.004	0.380 ± 0.020	0.278 ± 0.007
5	0.068 ± 0.000	0.623 ± 0.001	0.636 ± 0.025	0.210 ± 0.009	0.502 ± 0.056	0.621 ± 0.005	0.757 ± 0.034	0.658 ± 0.045
7	0.273 ± 0.016	0.884 ± 0.019	1.363 ± 0.032	0.498 ± 0.070	0.762 ± 0.005	1.267 ± 0.025	1.391 ± 0.147	1.048 ± 0.041
8	0.661 ± 0.024	1.774 ± 0.017	1.686 ± 0.052	1.054 ± 0.022	1.005 ± 0.091	1.837 ± 0.042	1.959 ± 0.038	1.557 ± 0.023
10	1.245 ± 0.060	2.005 ± 0.226	2.153 ± 0.133	1.486 ± 0.122	1.302 ± 0.014	2.266 ± 0.025	2.138 ± 0.172	1.840 ± 0.008
12	1.323 ± 0.075	2.055 ± 0.046	2.205 ± 0.068	1.518 ± 0.048	1.369 ± 0.018	2.303 ± 0.003	2.370 ± 0.053	1.929 ± 0.015
14	1.554 ± 0.130	2.114 ± 0.011	2.228 ± 0.082	1.600 ± 0.033	1.599 ± 0.052	2.361 ± 0.003	2.431 ± 0.079	2.087 ± 0.042
16	1.771 ± 0.071	2.167 ± 0.044	2.272 ± 0.092	1.633 ± 0.051	1.610 ± 0.072	2.369 ± 0.023	2.457 ± 0.052	2.236 ± 0.020
24	2.281 ± 0.005	2.204 ± 0.046	2.292 ± 0.087	1.739 ± 0.014	1.819 ± 0.058	2.441 ± 0.013	2.504 ± 0.014	2.361 ± 0.020
48	2.287 ± 0.000	2.333 ± 0.038	2.309 ± 0.075	2.047 ± 0.060	1.936 ± 0.061	2.538 ± 0.021	2.586 ± 0.013	2.575 ± 0.021
72	2.362 ± 0.006	2.408 ± 0.090	2.404 ± 0.039	2.281 ± 0.059	2.350 ± 0.095	2.740 ± 0.014	2.655 ± 0.007	2.737 ± 0.029
96	2.363 ± 0.025	2.235 ± 0.172	2.285 ± 0.082	2.175 ± 0.107	2.359 ± 0.101	2.581 ± 0.165	2.630 ± 0.023	2.708 ± 0.009

Table G-6 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 in mineral salt medium containing 2% wv^{-1} (11.1 mM) carbon source and $(NH_4)_2SO_4$ as nitrogen source at 37°C, 250 rpm (n = 3) (Figure 4.31).

Time	OD_{600}, *Enterobacter* sp. P2, 11.1 mM Glucose + $(NH_4)_2SO_4$				OD_{600}, *Burkholderia cepacia* P3, 11.1 mM Glucose + $(NH_4)_2SO_4$			
(hours)	Control	15 mM	75 mM	150 mM	Control	15 mM	75 mM	150 mM
0	0.006 ± 0.002	0.155 ± 0.003	0.177 ± 0.002	0.142 ± 0.012	0.023 ± 0.015	0.097 ± 0.002	0.097 ± 0.002	0.097 ± 0.002
2	0.015 ± 0.002	0.192 ± 0.006	0.182 ± 0.002	0.148 ± 0.009	0.039 ± 0.006	0.134 ± 0.002	0.158 ± 0.007	0.156 ± 0.003
5	0.068 ± 0.000	0.186 ± 0.019	0.196 ± 0.001	0.196 ± 0.003	0.502 ± 0.056	0.182 ± 0.006	0.250 ± 0.024	0.554 ± 0.042
7	0.273 ± 0.016	0.238 ± 0.005	0.417 ± 0.043	0.967 ± 0.015	0.762 ± 0.005	0.196 ± 0.001	0.597 ± 0.001	0.966 ± 0.020
8	0.661 ± 0.024	0.508 ± 0.005	1.027 ± 0.011	1.232 ± 0.031	1.005 ± 0.091	0.355 ± 0.002	1.185 ± 0.004	1.440 ± 0.016
10	1.245 ± 0.060	1.345 ± 0.023	1.481 ± 0.005	1.375 ± 0.049	1.302 ± 0.014	0.788 ± 0.001	1.485 ± 0.004	1.774 ± 0.018
12	1.323 ± 0.075	1.395 ± 0.009	1.472 ± 0.038	1.449 ± 0.004	1.369 ± 0.018	0.941 ± 0.037	1.763 ± 0.005	1.978 ± 0.014
14	1.554 ± 0.130	1.569 ± 0.034	1.457 ± 0.072	1.435 ± 0.028	1.599 ± 0.052	1.046 ± 0.039	1.948 ± 0.040	2.027 ± 0.019
16	1.771 ± 0.071	1.555 ± 0.087	1.471 ± 0.064	1.435 ± 0.035	1.610 ± 0.072	1.167 ± 0.016	1.978 ± 0.022	2.160 ± 0.039
24	2.281 ± 0.005	2.031 ± 0.011	1.772 ± 0.054	1.522 ± 0.070	1.819 ± 0.058	1.352 ± 0.035	2.169 ± 0.016	2.238 ± 0.068
48	2.287 ± 0.000	2.129 ± 0.019	1.852 ± 0.004	1.622 ± 0.025	1.936 ± 0.061	1.773 ± 0.026	2.352 ± 0.018	2.277 ± 0.044
72	2.362 ± 0.006	2.339 ± 0.018	2.171 ± 0.050	1.782 ± 0.020	2.350 ± 0.095	2.358 ± 0.022	2.455 ± 0.126	2.358 ± 0.045
96	2.363 ± 0.025	2.303 ± 0.015	2.034 ± 0.003	1.738 ± 0.013	2.359 ± 0.101	2.274 ± 0.019	2.398 ± 0.046	2.259 ± 0.031

Table G-7 Growth of *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 in mineral salt medium containing 2% wv^{-1} (11.1 mM) carbon source and CH_4N_2O as nitrogen source at 37°C, 250 rpm (n = 3) (Figure 4.32).

Time	OD_{600}, *Enterobacter* sp. P2, 11.1 mM Glucose + CH_4N_2O				OD_{600}, *Burkholderia cepacia* P3, 11.1 mM Glucose + CH_4N_2O			
(hours)	Control	15 mM	75 mM	150 mM	Control	15 mM	75 mM	150 mM
0	0.006 ± 0.002	0.129 ± 0.000	0.142 ± 0.002	0.147 ± 0.001	0.023 ± 0.015	0.024 ± 0.001	0.085 ± 0.003	0.051 ± 0.005
2	0.015 ± 0.002	0.154 ± 0.003	0.150 ± 0.004	0.165 ± 0.003	0.039 ± 0.006	0.124 ± 0.002	0.195 ± 0.003	0.091 ± 0.006
5	0.068 ± 0.000	0.123 ± 0.010	0.124 ± 0.004	0.153 ± 0.002	0.502 ± 0.056	0.163 ± 0.002	0.239 ± 0.006	0.116 ± 0.006
7	0.273 ± 0.016	0.273 ± 0.038	0.138 ± 0.002	0.217 ± 0.002	0.762 ± 0.005	0.241 ± 0.008	0.422 ± 0.014	0.367 ± 0.002
8	0.661 ± 0.024	0.536 ± 0.037	0.228 ± 0.006	0.474 ± 0.012	1.005 ± 0.091	0.472 ± 0.002	0.686 ± 0.001	0.456 ± 0.003
10	1.245 ± 0.060	1.072 ± 0.100	0.741 ± 0.009	1.237 ± 0.029	1.302 ± 0.014	0.728 ± 0.008	1.035 ± 0.000	0.844 ± 0.013
12	1.323 ± 0.075	1.415 ± 0.010	1.323 ± 0.010	1.418 ± 0.015	1.369 ± 0.018	1.049 ± 0.034	1.338 ± 0.013	1.055 ± 0.043
14	1.554 ± 0.130	1.618 ± 0.014	1.421 ± 0.015	1.389 ± 0.013	1.599 ± 0.052	1.157 ± 0.025	1.563 ± 0.004	1.148 ± 0.019
16	1.771 ± 0.071	1.848 ± 0.027	1.520 ± 0.018	1.447 ± 0.004	1.610 ± 0.072	1.246 ± 0.034	1.829 ± 0.021	1.369 ± 0.023
24	2.281 ± 0.005	1.959 ± 0.034	1.717 ± 0.022	1.457 ± 0.030	1.819 ± 0.058	1.548 ± 0.021	1.940 ± 0.014	1.506 ± 0.031
48	2.287 ± 0.000	2.014 ± 0.013	1.780 ± 0.017	1.610 ± 0.025	1.936 ± 0.061	1.777 ± 0.011	2.235 ± 0.003	1.809 ± 0.035
72	2.362 ± 0.006	2.077 ± 0.012	1.802 ± 0.002	1.902 ± 0.006	2.350 ± 0.095	2.235 ± 0.003	2.474 ± 0.021	1.962 ± 0.030
96	2.363 ± 0.025	2.086 ± 0.089	1.769 ± 0.081	1.918 ± 0.007	2.359 ± 0.101	2.235 ± 0.021	2.429 ± 0.012	1.942 ± 0.069

Table G-8 Biosurfactant production of the two bacterial isolates in mineral salt medium containing 2% wv^{-1} (11.1 mM) carbon sources and varies types and concentration of nitrogen source at 37°C, 250 rpm (n = 3) (Figure 4.33).

Productive condition in the presence of 2% wv^{-1} glucose	**Glycolipid concentration ($g.l^{-1}$)**	
	***Enterobacter* sp. P2**	***B. cepacia* P3**
Culture medium (MSM)	17.49 ± 0.338	37.01 ± 0.118
MSM + 15 mM $NaNO_3$	13.70 ± 0.467	37.20 ± 0.134
MSM + 75 mM $NaNO_3$	17.58 ± 0.044	46.61 ± 0.414
MSM + 150 mM $NaNO_3$	14.58 ± 0.100	46.91 ± 0.148
MSM + 15 mM $(NH_4)_2SO_4$	8.90 ± 0.073	28.40 ± 0.770
MSM + 75 mM $(NH_4)_2SO_4$	10.48 ± 0.542	33.93 ± 0.189
MSM + 150 mM $(NH_4)_2SO_4$	9.60 ± 0.303	30.89 ± 0.167
MSM + 15 mM CH_4N_2O	9.77 ± 0.002	16.09 ± 0.861
MSM + 75 mM CH_4N_2O	14.27 ± 0.111	31.61 ± 1.053
MSM + 150 mM CH_4N_2O	10.89 ± 0.089	31.02 ± 0.218

Table G-9 Growth of *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 in mineral salt medium containing 2% wv^{-1} (11.1 mM) glucose as carbon source and $NaNO_3$ as nitrogen source at 37°C, 250 rpm (n = 3)

Time (hours)	OD_{600}, *Enterobacter* sp. P2, 11.1 mM Glucose + $NaNO_3$				OD_{600}, *Burkholderia cepacia* P3, 11.1 mM Glucose + $NaNO_3$			
	Control	15 mM	75 mM	150 mM	Control	15 mM	75 mM	150 mM
0	0.006 ± 0.002	0.048 ± 0.007	0.004 ± 0.003	0.011 ± 0.014	0.023 ± 0.015	0.004 ± 0.002	0.042 ± 0.007	0.052 ± 0.036
2	0.015 ± 0.002	0.062 ± 0.006	0.033 ± 0.028	0.139 ± 0.032	0.039 ± 0.006	0.033 ± 0.009	0.145 ± 0.021	0.144 ± 0.013
5	0.068 ± 0.000	0.093 ± 0.003	0.066 ± 0.005	0.165 ± 0.022	0.502 ± 0.056	0.373 ± 0.084	0.252 ± 0.022	0.155 ± 0.001
7	0.273 ± 0.016	0.143 ± 0.027	0.080 ± 0.010	0.222 ± 0.015	0.762 ± 0.005	0.518 ± 0.051	0.278 ± 0.018	0.245 ± 0.015
8	0.661 ± 0.024	0.237 ± 0.004	0.123 ± 0.006	0.272 ± 0.027	1.005 ± 0.091	0.658 ± 0.069	0.311 ± 0.011	0.313 ± 0.012
10	1.245 ± 0.060	0.271 ± 0.027	0.271 ± 0.025	0.312 ± 0.012	1.302 ± 0.014	0.739 ± 0.021	0.362 ± 0.008	0.341 ± 0.009
12	1.323 ± 0.075	0.622 ± 0.049	0.603 ± 0.026	0.356 ± 0.007	1.369 ± 0.018	0.852 ± 0.043	0.375 ± 0.021	0.406 ± 0.001
14	1.554 ± 0.130	0.793 ± 0.124	0.890 ± 0.110	0.405 ± 0.004	1.599 ± 0.052	1.093 ± 0.085	0.390 ± 0.022	0.446 ± 0.026
16	1.771 ± 0.071	0.964 ± 0.059	1.105 ± 0.102	0.435 ± 0.040	1.610 ± 0.072	1.147 ± 0.023	0.427 ± 0.024	0.486 ± 0.007
24	2.281 ± 0.005	1.199 ± 0.104	1.370 ± 0.048	0.458 ± 0.036	1.819 ± 0.058	1.282 ± 0.045	0.458 ± 0.031	0.500 ± 0.004
48	2.287 ± 0.000	1.399 ± 0.028	1.522 ± 0.076	0.493 ± 0.024	1.936 ± 0.061	1.351 ± 0.008	0.509 ± 0.008	0.538 ± 0.028
72	2.362 ± 0.006	1.478 ± 0.026	1.708 ± 0.060	0.519 ± 0.059	2.350 ± 0.095	1.361 ± 0.005	0.515 ± 0.018	0.565 ± 0.031
96	2.363 ± 0.025	1.472 ± 0.024	1.689 ± 0.107	0.617 ± 0.009	2.359 ± 0.101	1.348 ± 0.014	0.525 ± 0.003	0.609 ± 0.009

Table G-10 Growth of *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 in mineral salt medium containing 4% wv^{-1} (44.4 mM) glucose as carbon source and $NaNO_3$ as nitrogen source at 37°C, 250 rpm (n = 3)

Time	OD_{600}, *Enterobacter* sp. P2, 44.4 mM Glucose + $NaNO_3$				OD_{600}, *Burkholderia cepacia* P3, 44.4 mM Glucose + $NaNO_3$			
(hours)	Control	15 mM	75 mM	150 mM	Control	15 mM	75 mM	150 mM
0	0.006 ± 0.002	0.040 ± 0.001	0.013 ± 0.001	0.021 ± 0.015	0.023 ± 0.015	0.029 ± 0.009	0.036 ± 0.000	0.019 ± 0.013
2	0.015 ± 0.002	0.068 ± 0.009	0.138 ± 0.032	0.049 ± 0.010	0.039 ± 0.006	0.139 ± 0.024	0.091 ± 0.043	0.163 ± 0.027
5	0.068 ± 0.000	0.091 ± 0.003	0.171 ± 0.039	0.128 ± 0.005	0.502 ± 0.056	0.271 ± 0.025	0.243 ± 0.107	0.192 ± 0.041
7	0.273 ± 0.016	0.124 ± 0.002	0.239 ± 0.000	0.194 ± 0.004	0.762 ± 0.005	0.397 ± 0.037	0.665 ± 0.148	0.239 ± 0.001
8	0.661 ± 0.024	0.249 ± 0.021	0.397 ± 0.050	0.245 ± 0.016	1.005 ± 0.091	0.488 ± 0.047	1.005 ± 0.049	0.305 ± 0.015
10	1.245 ± 0.060	0.299 ± 0.059	0.892 ± 0.042	0.289 ± 0.008	1.302 ± 0.014	0.556 ± 0.020	1.600 ± 0.072	0.337 ± 0.015
12	1.323 ± 0.075	0.465 ± 0.131	1.416 ± 0.188	0.317 ± 0.005	1.369 ± 0.018	0.623 ± 0.022	1.695 ± 0.051	0.350 ± 0.016
14	1.554 ± 0.130	0.594 ± 0.104	1.665 ± 0.101	0.358 ± 0.011	1.599 ± 0.052	0.620 ± 0.005	1.913 ± 0.031	0.369 ± 0.028
16	1.771 ± 0.071	0.793 ± 0.175	1.963 ± 0.059	0.428 ± 0.006	1.610 ± 0.072	0.714 ± 0.011	2.069 ± 0.052	0.381 ± 0.027
24	2.281 ± 0.005	1.040 ± 0.233	2.067 ± 0.047	0.481 ± 0.025	1.819 ± 0.058	0.726 ± 0.003	2.183 ± 0.042	0.399 ± 0.023
48	2.287 ± 0.000	1.327 ± 0.078	2.251 ± 0.015	0.495 ± 0.018	1.936 ± 0.061	0.793 ± 0.000	2.336 ± 0.032	0.414 ± 0.017
72	2.362 ± 0.006	1.498 ± 0.082	2.460 ± 0.031	0.533 ± 0.010	2.350 ± 0.095	0.809 ± 0.007	2.560 ± 0.011	0.481 ± 0.054
96	2.363 ± 0.025	1.494 ± 0.079	2.441 ± 0.034	0.528 ± 0.012	2.359 ± 0.101	0.802 ± 0.002	2.539 ± 0.002	0.468 ± 0.052

Table G-11 Growth of *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 in mineral salt medium containing 15% wv^{-1} (83.3 mM) glucose as carbon source and $NaNO_3$ as nitrogen source at 37°C, 250 rpm (n = 3)

Time (hours)	OD_{600}, *Enterobacter* sp. P2, 83.3 mM Glucose + $NaNO_3$				OD_{600}, *Burkholderia cepacia* P3, 83.3 mM Glucose + $NaNO_3$			
	Control	15 mM	75 mM	150 mM	Control	15 mM	75 mM	150 mM
0	0.006 ± 0.002	0.016 ± 0.010	0.006 ± 0.002	0.023 ± 0.001	0.023 ± 0.015	0.032 ± 0.001	0.002 ± 0.001	0.043 ± 0.006
2	0.015 ± 0.002	0.029 ± 0.013	0.055 ± 0.021	0.046 ± 0.020	0.039 ± 0.006	0.070 ± 0.026	0.003 ± 0.001	0.068 ± 0.007
5	0.068 ± 0.000	0.056 ± 0.016	0.148 ± 0.021	0.102 ± 0.001	0.502 ± 0.056	0.089 ± 0.016	0.005 ± 0.001	0.085 ± 0.008
7	0.273 ± 0.016	0.117 ± 0.051	0.163 ± 0.028	0.184 ± 0.001	0.762 ± 0.005	0.104 ± 0.019	0.007 ± 0.001	0.097 ± 0.005
8	0.661 ± 0.024	0.158 ± 0.048	0.212 ± 0.022	0.236 ± 0.000	1.005 ± 0.091	0.122 ± 0.016	0.121 ± 0.007	0.116 ± 0.014
10	1.245 ± 0.060	0.184 ± 0.044	1.135 ± 0.259	0.283 ± 0.005	1.302 ± 0.014	0.141 ± 0.017	0.131 ± 0.008	0.140 ± 0.015
12	1.323 ± 0.075	0.390 ± 0.071	1.300 ± 0.101	0.317 ± 0.016	1.369 ± 0.018	0.156 ± 0.028	0.141 ± 0.010	0.170 ± 0.007
14	1.554 ± 0.130	0.645 ± 0.041	1.389 ± 0.034	0.419 ± 0.003	1.599 ± 0.052	0.175 ± 0.022	0.154 ± 0.015	0.194 ± 0.007
16	1.771 ± 0.071	0.872 ± 0.017	1.494 ± 0.056	0.431 ± 0.010	1.610 ± 0.072	0.194 ± 0.015	0.171 ± 0.002	0.221 ± 0.020
24	2.281 ± 0.005	1.114 ± 0.105	1.562 ± 0.036	0.457 ± 0.003	1.819 ± 0.058	0.210 ± 0.010	0.185 ± 0.005	0.220 ± 0.010
48	2.287 ± 0.000	1.328 ± 0.153	1.600 ± 0.046	0.527 ± 0.002	1.936 ± 0.061	0.221 ± 0.011	0.188 ± 0.004	0.234 ± 0.018
72	2.362 ± 0.006	1.544 ± 0.041	1.643 ± 0.062	0.556 ± 0.014	2.350 ± 0.095	0.232 ± 0.006	0.199 ± 0.006	0.248 ± 0.014
96	2.363 ± 0.025	1.538 ± 0.042	1.638 ± 0.065	0.570 ± 0.005	2.359 ± 0.101	0.226 ± 0.007	0.198 ± 0.006	0.244 ± 0.010

Table G-12 Biosurfactant production of *Enterobacter* sp. P2 and *B. cepacia* P3 in the combination of carbon or nitrogen sources at 37°C, 250 rpm. The data were means from three independent experiments with vertical bars representing standard errors of the means (n=3) (Figure 4.34).

Productive condition	**Glycolipid concentration ($g.l^{-1}$)**	
	***Enterobacter* sp. P2**	***B. cepacia* P3**
Culture medium	17.49 ± 0.338	37.01 ± 0.118
11.1 mM Glucose + 15 mM $NaNO_3$	4.11 ± 0.007	16.09 ± 0.861
11.1 mM Glucose + 75 mM $NaNO_3$	4.68 ± 0.066	7.65 ± 0.037
11.1 mM Glucose + 150 mM $NaNO_3$	2.88 ± 0.048	3.30 ± 0.171
44.4 mM Glucose + 15 mM $NaNO_3$	9.67 ± 0.042	28.40 ± 0.770
44.4 mM Glucose + 75 mM $NaNO_3$	18.72 ± 0.071	40.67 ± 0.992
44.4 mM Glucose + 150 mM $NaNO_3$	5.52 ± 0.530	16.24 ± 0.176
83.3 mM Glucose + 15 mM $NaNO_3$	5.02 ± 0.469	No detect
83.3 mM Glucose + 75 mM $NaNO_3$	4.06 ± 0.067	No detect
83.3 mM Glucose + 150 mM $NaNO_3$	3.44 ± 0.006	No detect

Various types oil (e.g. sunflower oil, olive oil, soybean oil, and diesel oil) were supplemented during growth to determine if addition of oil improve biosurfactant production. The organism grew in MSM (2% wv^{-1} glucose) with oil as supplemented carbon sources and produced biosurfactant.

Table G-13 Growth of *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 in mineral salt medium containing 2% wv^{-1} glucose as carbon source supplemented with various type of oil (2% vv^{-1}) as an additional carbon source at 37°C, 250 rpm (n = 3) (Figure 4.35).

Time	OD_{600}, *Enterobacter* sp. P2, 11.1 mM Glucose + 2% vv^{-1} oil					OD_{600}, *Burkholderia cepacia* P3, 11.1 mM Glucose + 2% vv^{-1} oil				
(hours)	Control	Sunflower oil	Olive oil	Soybean oil	Diesel oil	Control	Sunflower oil	Olive oil	Soybean oil	Diesel oil
0	0.006 ± 0.002	0.008 ± 0.000	0.005 ± 0.004	0.005 ± 0.004	0.008 ± 0.001	0.023 ± 0.015	0.002 ± 0.003	0.001 ± 0.000	0.003 ± 0.002	0.027 ± 0.025
2	0.015 ± 0.002	0.142 ± 0.043	0.269 ± 0.026	0.124 ± 0.003	0.129 ± 0.022	0.039 ± 0.006	0.342 ± 0.024	0.265 ± 0.043	0.155 ± 0.004	0.051 ± 0.039
5	0.068 ± 0.000	0.236 ± 0.039	0.463 ± 0.013	0.338 ± 0.029	0.245 ± 0.031	0.502 ± 0.056	0.436 ± 0.028	0.617 ± 0.040	0.191 ± 0.009	0.142 ± 0.013
7	0.273 ± 0.016	0.530 ± 0.027	1.135 ± 0.042	0.657 ± 0.034	0.256 ± 0.033	0.762 ± 0.005	0.970 ± 0.031	0.701 ± 0.041	0.558 ± 0.024	0.201 ± 0.031
8	0.661 ± 0.024	1.431 ± 0.122	1.571 ± 0.021	1.250 ± 0.040	0.891 ± 0.013	1.005 ± 0.091	1.456 ± 0.025	1.055 ± 0.004	0.753 ± 0.008	0.330 ± 0.063
10	1.245 ± 0.060	2.101 ± 0.115	1.759 ± 0.020	1.568 ± 0.024	1.452 ± 0.029	1.302 ± 0.014	2.321 ± 0.034	1.373 ± 0.011	1.056 ± 0.003	0.471 ± 0.027
12	1.323 ± 0.075	2.345 ± 0.012	1.921 ± 0.050	1.741 ± 0.024	1.597 ± 0.002	1.369 ± 0.018	2.561 ± 0.026	1.753 ± 0.003	1.253 ± 0.028	0.525 ± 0.063
14	1.554 ± 0.130	2.471 ± 0.012	2.068 ± 0.017	1.830 ± 0.025	1.769 ± 0.016	1.599 ± 0.052	2.658 ± 0.002	1.857 ± 0.008	1.366 ± 0.010	0.687 ± 0.034
16	1.771 ± 0.071	2.565 ± 0.005	2.268 ± 0.030	1.850 ± 0.043	1.941 ± 0.020	1.610 ± 0.072	2.770 ± 0.012	2.063 ± 0.005	1.658 ± 0.025	0.824 ± 0.099
24	2.281 ± 0.005	2.593 ± 0.011	2.379 ± 0.016	1.924 ± 0.032	2.026 ± 0.018	1.819 ± 0.058	2.851 ± 0.002	2.346 ± 0.037	1.945 ± 0.004	1.077 ± 0.033
48	2.287 ± 0.000	2.662 ± 0.006	2.586 ± 0.020	2.054 ± 0.037	2.094 ± 0.002	1.936 ± 0.061	2.886 ± 0.010	2.562 ± 0.026	2.146 ± 0.021	1.232 ± 0.129
72	2.362 ± 0.006	2.761 ± 0.005	2.656 ± 0.005	2.094 ± 0.004	2.561 ± 0.029	2.350 ± 0.095	3.038 ± 0.016	2.665 ± 0.019	2.240 ± 0.025	1.981 ± 0.023
96	2.363 ± 0.025	2.661 ± 0.010	2.681 ± 0.007	2.080 ± 0.015	2.502 ± 0.067	2.359 ± 0.101	3.025 ± 0.049	2.629 ± 0.024	2.200 ± 0.009	1.963 ± 0.027

Table G-14 Effect of the supplemented carbon sources on glycolipid production by *Enterobacter* sp. P2 and *B. cepacia* P3 measured at 31°C (n = 4) (Figure 4.36).

2% vv^{-1} supplemented carbon source	**Glycolipid concentration ($g.l^{-1}$)**	
	***Enterobacter* sp. P2**	***B. cepacia* P3**
Glucose alone	17.49 ± 0.338	37.01 ± 0.118
Sunflowers oil	13.72 ± 0.235	55.08 ± 0.234
Olive oil	13.72 ± 0.451	44.68 ± 0.026
Soy bean oil	14.11 ± 0.360	23.30 ± 0.178
Diesel oil	11.74 ± 0.126	27.93 ± 0.283

Table G-15 Growth of *Enterobacter* sp. P2 and *B. cepacia* P3 which was grown in mineral salt medium supplemented with 2% wv^{-1} glucose as carbon source at 30°C, 37°C and 45°C cannot detected. Each point represented the mean and standard deviation of triplicate samples (Figure 4.37).

Time	**OD_{600}, *Enterobacter* sp. P2**			**OD_{600}, *Burkholderia cepacia* P3**		
(hours)	**30°C**	**37°C**	**45°C**	**30°C**	**37°C**	**45°C**
0	0.010 ± 0.001	0.006 ± 0.002	ND	0.055 ± 0.006	0.023 ± 0.015	ND
2	0.118 ± 0.002	0.015 ± 0.002	ND	0.101 ± 0.005	0.039 ± 0.006	ND
5	0.252 ± 0.005	0.068 ± 0.000	ND	0.133 ± 0.002	0.502 ± 0.056	ND
7	0.414 ± 0.017	0.273 ± 0.016	ND	0.181 ± 0.001	0.762 ± 0.005	ND
8	0.685 ± 0.000	0.661 ± 0.024	ND	0.193 ± 0.004	1.005 ± 0.091	ND
10	1.080 ± 0.015	1.245 ± 0.060	ND	0.731 ± 0.063	1.302 ± 0.014	ND
12	1.148 ± 0.041	1.323 ± 0.075	ND	0.861 ± 0.006	1.369 ± 0.018	ND
14	1.305 ± 0.004	1.554 ± 0.130	ND	1.032 ± 0.027	1.599 ± 0.052	ND
16	1.480 ± 0.055	1.771 ± 0.071	ND	1.426 ± 0.002	1.610 ± 0.072	ND
24	2.021 ± 0.012	2.281 ± 0.005	ND	1.537 ± 0.002	1.819 ± 0.058	ND
48	2.145 ± 0.007	2.287 ± 0.000	ND	1.816 ± 0.012	1.936 ± 0.061	ND
72	2.207 ± 0.003	2.362 ± 0.006	ND	2.031 ± 0.038	2.350 ± 0.095	ND
96	2.202 ± 0.003	2.363 ± 0.025	ND	2.104 ± 0.004	2.359 ± 0.101	ND

ND = no detect

Table G-16 Glycolipid productions obtained from growth of *Enterobacter* sp. P2 and *B. cepacia* P3 on 2% wv^{-1} glucose as carbon source for 72 hours at 30°C and 37°C, respectively. Each point represented the mean and standard deviation of triplicate samples (Figure 4.38).

Condition at temperature (°C)	**Glycolipid concentration ($g.l^{-1}$)**	
	***Enterobacter* sp. P2**	***B. cepacia* P3**
30°C	0.19 ± 0.002	31.48 ± 0.900
37°C	17.49 ± 0.338	37.01 ± 0.118
45°C	ND	ND

ND = no detect

For growth studies and biosurfactant production at different NaCl (0.1, 0.5, 1.0 and 2.0% wv^{-1} or 17.1, 85.5, 171.0 and 342.0mM) concentrations and pH values (4.5–10.5), the NaCl concentration and pH of the medium were adjusted accordingly. Growth studies were done using 2% wv^{-1} glucose as the carbon source. Experiments were done in triplicate and the results reported are averages of three independent experiments.

Table G-17 Growth of *Enterobacter* sp. P2 and *Burkholderia cepacia* P3 in 2% wv^{-1} glucose as carbon source which various concentrations of salt: the control, 0.1% wv^{-1} (17.1 mM) NaCl ,0.5% wv^{-1} (85.5 mM) NaCl, 1.0% wv^{-1} (171.0 mM) NaCl and 2.0% wv^{-1} (342.0 mM) NaCl and range of pH values (4.5–10.5). Each point represented the mean and standard deviation of triplicate samples (Figure 4.39).

Time	OD_{600}, *Enterobacter* sp. P2, 11.1 mM Glucose + NaCl					OD_{600}, *Burkholderia cepacia* P3, 11.1 mM Glucose + NaCl				
(hours)	Control	17.1 mM	85.5 mM	171.0 mM	342.0 mM	Control	17.1 mM	85.5 mM	171.0 mM	342.0 mM
0	0.006 ± 0.002	0.008 ± 0.001	0.003 ± 0.001	0.004 ± 0.000	0.003 ± 0.002	0.023 ± 0.015	0.045 ± 0.009	0.060 ± 0.001	0.083 ± 0.002	0.077 ± 0.010
2	0.015 ± 0.002	0.017 ± 0.004	0.031 ± 0.002	0.007 ± 0.001	0.008 ± 0.004	0.039 ± 0.006	0.058 ± 0.003	0.122 ± 0.002	0.085 ± 0.003	0.090 ± 0.001
5	0.068 ± 0.000	0.061 ± 0.006	0.036 ± 0.001	0.008 ± 0.005	0.014 ± 0.002	0.502 ± 0.056	0.083 ± 0.006	0.132 ± 0.002	0.161 ± 0.012	0.080 ± 0.000
7	0.273 ± 0.016	0.076 ± 0.012	0.061 ± 0.020	0.114 ± 0.001	0.019 ± 0.006	0.762 ± 0.005	0.124 ± 0.010	0.144 ± 0.000	0.425 ± 0.001	0.107 ± 0.011
8	0.661 ± 0.024	0.094 ± 0.018	0.085 ± 0.016	0.119 ± 0.006	0.024 ± 0.006	1.005 ± 0.091	0.236 ± 0.003	0.570 ± 0.000	0.545 ± 0.025	0.193 ± 0.004
10	1.245 ± 0.060	0.171 ± 0.089	0.140 ± 0.078	0.127 ± 0.003	0.033 ± 0.007	1.302 ± 0.014	0.264 ± 0.028	1.019 ± 0.007	0.610 ± 0.049	0.360 ± 0.001
12	1.323 ± 0.075	0.395 ± 0.173	0.182 ± 0.063	0.133 ± 0.005	0.039 ± 0.005	1.369 ± 0.018	0.631 ± 0.003	1.198 ± 0.000	0.633 ± 0.026	0.364 ± 0.006
14	1.554 ± 0.130	0.722 ± 0.174	0.335 ± 0.135	0.135 ± 0.005	0.056 ± 0.010	1.599 ± 0.052	0.713 ± 0.021	1.013 ± 0.005	0.737 ± 0.007	0.367 ± 0.006
16	1.771 ± 0.071	1.038 ± 0.160	0.390 ± 0.049	0.136 ± 0.005	0.073 ± 0.006	1.610 ± 0.072	0.939 ± 0.024	1.067 ± 0.039	0.838 ± 0.001	0.380 ± 0.009
24	2.281 ± 0.005	1.279 ± 0.085	0.412 ± 0.050	0.139 ± 0.003	0.079 ± 0.004	1.819 ± 0.058	1.166 ± 0.049	1.212 ± 0.001	0.991 ± 0.010	0.392 ± 0.009
48	2.287 ± 0.000	1.418 ± 0.097	0.445 ± 0.014	0.140 ± 0.002	0.081 ± 0.005	1.936 ± 0.061	1.407 ± 0.076	1.346 ± 0.001	1.087 ± 0.057	0.398 ± 0.006
72	2.362 ± 0.006	1.613 ± 0.138	0.479 ± 0.010	0.144 ± 0.002	0.086 ± 0.006	2.350 ± 0.095	1.532 ± 0.083	1.471 ± 0.001	1.230 ± 0.006	0.406 ± 0.005
96	2.363 ± 0.025	1.798 ± 0.083	0.475 ± 0.012	0.143 ± 0.002	0.084 ± 0.007	2.359 ± 0.101	1.723 ± 0.047	1.396 ± 0.000	1.124 ± 0.000	0.401 ± 0.003

Table G-18 Effect of NaCl on production of *Enterobacter* sp. P2 and *B. cepacia* P3. The data were means from three independent experiments with vertical bars representing standard errors of the means (n=3) (Figure 4.40).

Productive condition in the presence of 2% wv^{-1} glucose + NaCl	**Glycolipid concentration ($g.l^{-1}$)**	
	***Enterobacter* sp. P2**	***B. cepacia* P3**
Control	17.49 ± 0.338	37.01 ± 0.118
0.1% wv^{-1} (17.1 mM) NaCl	2.24 ± 0.023	11.88 ± 0.085
0.5% wv^{-1} (85.5 mM) NaCl	0.18 ± 0.003	2.55 ± 0.008
1.0% wv^{-1} (171.0 mM) NaCl	0.07 ± 0.008	0.86 ± 0.001
2.0% wv^{-1} (342.0 mM) NaCl	0.00 ± 0.000	0.00 ± 0.000

BIOGRAPHY

Miss Hathairath T.Wattanaphon was born on December 18, in Bangkok province, Thailand. She received Bachelor's Degree in Biochemistry, Faculty of science, Chulalongkorn University in 2004. She pursed his Master degree study in the Biotechnology Program, Faculty of science, Chulalongkorn University, Bangkok, Thailand in June 2004. She finished Master Degree of Science in Biotechnology Program in October 2006.