

Supplementary Figure Legends

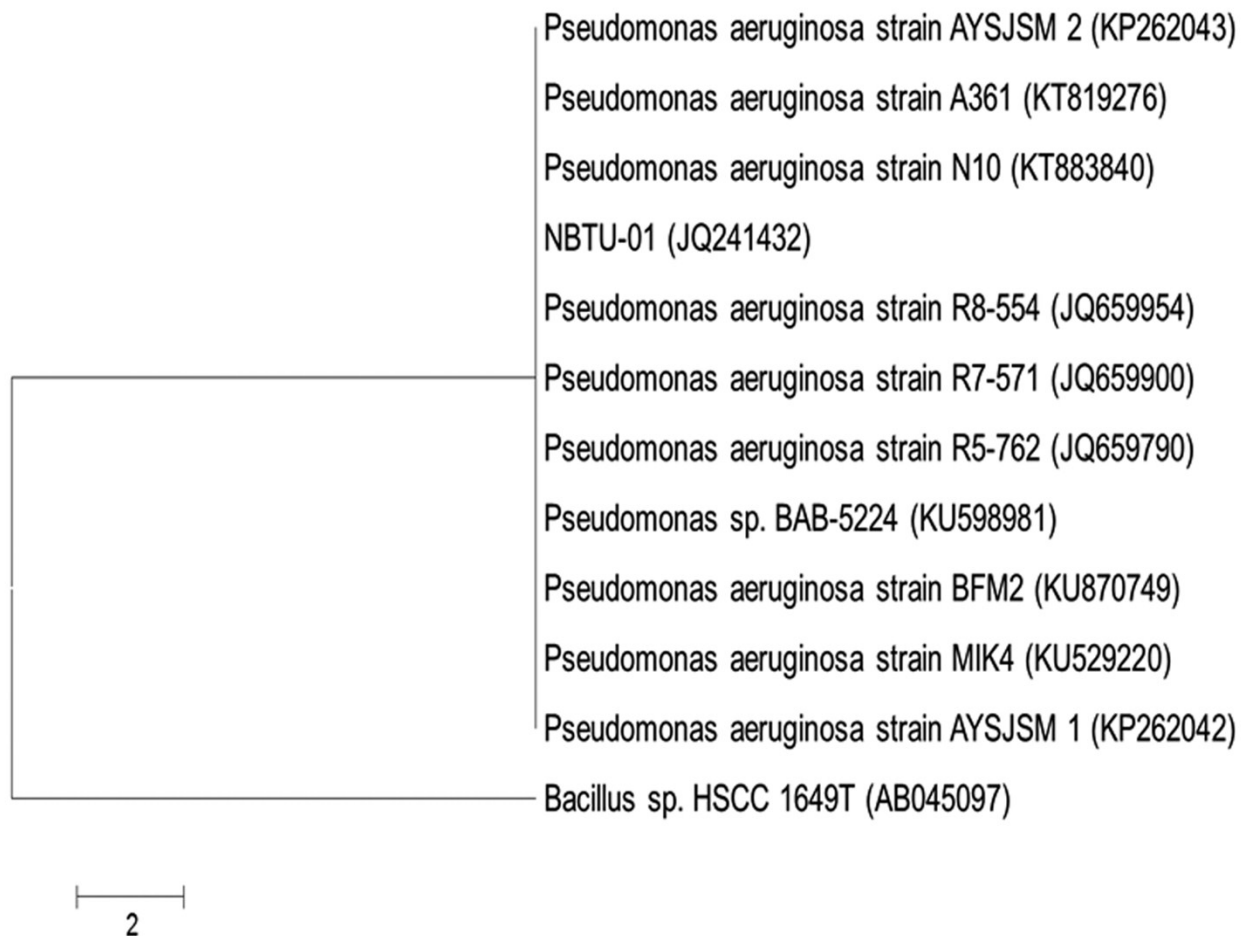
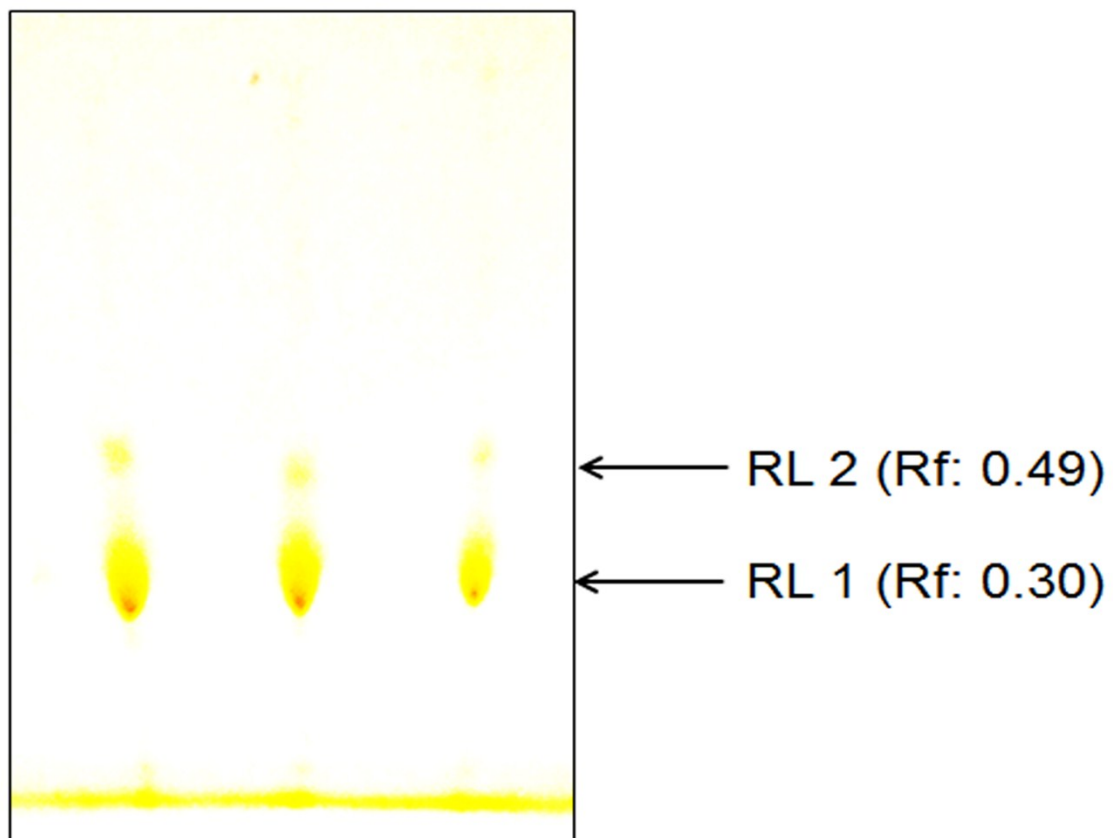


Fig. S1. Phylogenetic relationships of strain NBTU-01 and other closely related *Pseudomonas* species based on 16S rDNA sequencing. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 683 positions in the final dataset. The tree was generated using the neighbor-joining method and the sequence from *Bacillus* sp. HSCC 1649 T (Accession no. AB045097) was considered as out-group. The stability of tree obtained from the above cluster analyses was assessed by using BOOTSTRAP program in sets of 1,000 resamplings (MEGA 6) and percentage values are given at the nodes.



50:50 (Chloroform: methanol, (v/v) eluted fraction)

Fig. S2. TLC analysis of chloroform: methanol (50:50 v/v) eluted biosurfactant fraction from Silica gel G column. The colour in TLC plate was developed by iodine vapor staining method. The analysis was performed in triplicate to assure the reproducibility. RL1 and RL2 represent dirhamnolipid and monorhamnolipid, respectively.

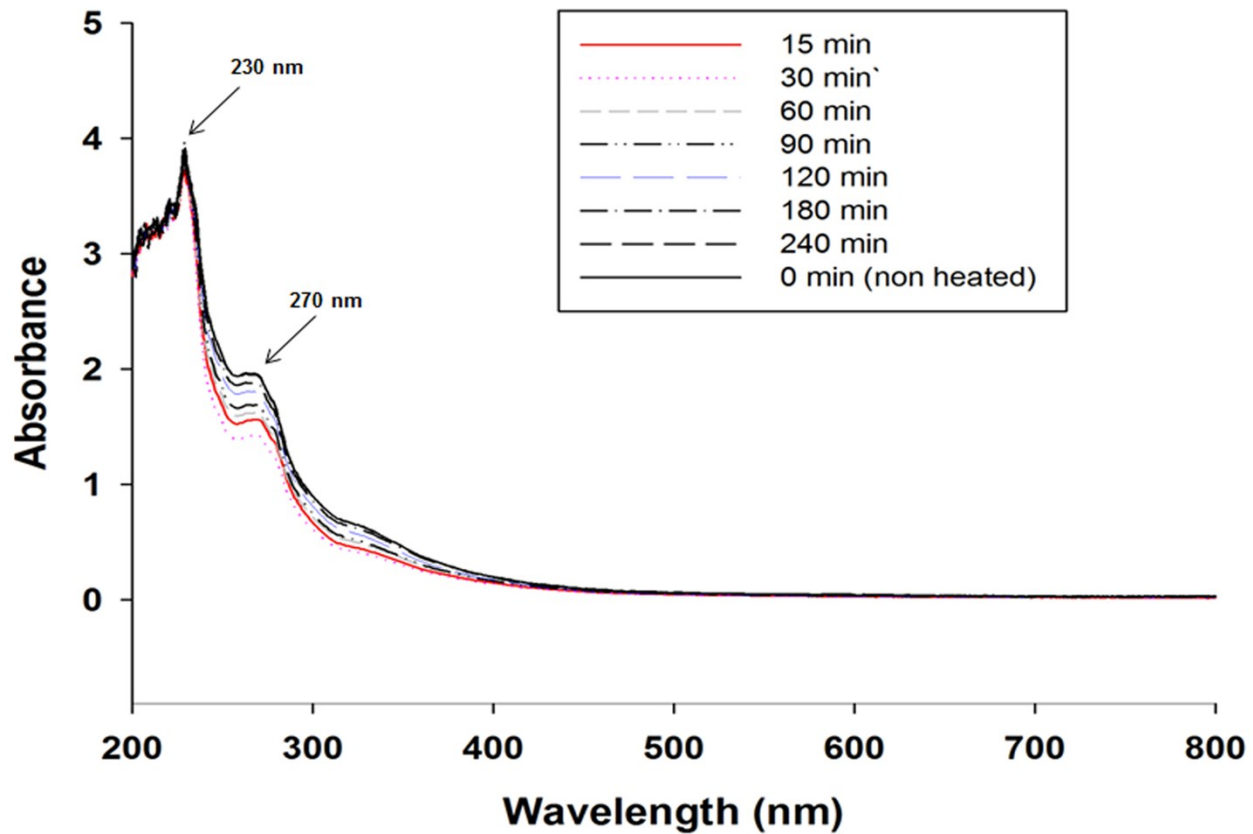


Fig. S3. UV-Vis spectral analysis of non-heated (control) and heated rhamnolipid biosurfactant (100 $\mu\text{g/ml}$) at 100 $^{\circ}\text{C}$ for different time intervals. The data represents average of three scans.

Supplementary Tables

Table S1. Yield of bacterial dry biomass, protein content and surface tension of culture medium supplemented with 1% (v/v) glycerol or hexadecane in M9 medium post 96 h of inoculation at 37 °C. Values are mean \pm S.D. of three determinations.

Bacterial Isolates	Glycerol			Hexadecane		
	Dry biomass (g l ⁻¹)	Protein content (mg l ⁻¹)	Surface tension (mNm ⁻¹)	Dry biomass (g l ⁻¹)	Protein content (mg l ⁻¹)	Surface tension (mNm ⁻¹)
NBTU-01	3.4 \pm 0.1	664.0 \pm 2.0	31.5 \pm 0.29*	3.1 \pm 0.3	38.4 \pm 0.2	34.2 \pm 0.4*
G#85	0.25 \pm 0.01	3.2 \pm 0.3	58.9 \pm 0.2	1.2 \pm 0.01	8.8 \pm 0.23	38.0 \pm 0.6
G#207	0.5 \pm 0.08	0.2 \pm 0.003	64.1 \pm 0.8	3.5 \pm 0.2	15.2 \pm 0.9	53.3 \pm 0.2
G#191	0.55 \pm 0.1	0.6 \pm 0.01	61.1 \pm 0.9	2.1 \pm 0.65	1.8 \pm 0.02	51.3 \pm 1.1
W2	0.8 \pm 0.1	0.8 \pm 0.2	62.6 \pm 1.1	2.8 \pm 0.35	8.0 \pm 0.3	54.2 \pm 0.55
Control	ND	ND	68.0 \pm 2.1	ND	ND	68.7 \pm 1.2

ND: Not determined

Statistical analysis: *p < 0.001 with respect to control value.

Table S2. Morphological, phenotypical and biochemical properties of *P. aeruginosa* strain NBTU-01

Morphological and phenotypic characteristics	
Parameters	Observations
Size	Small
Pigment	Greenish in color
Form	Rod
Margin	Umbonate elevation
Transparency	Opaque
Shape	Regular
Surface morphology	Smooth, slightly mucoid
Biochemical characteristics	
Catalase	+
Oxidase	+
Citrate utilization	+
Urease	+
Gelatin hydrolysis	+
Starch hydrolysis	-
Lipid hydrolysis	-
Casein hydrolysis	-
MR-VP	-

+ indicate presence of activity, - indicates absence of activity

Table S3. A composition of the type of rhamnolipids produced by different *P. aeruginosa* strains during growth on glycerol supplemented M9 medium. ^a Predominant rhamnolipids in the sample.

Mono-rhamnolipid	Di-rhamnolipid	References
Main carbon source= glycerol (8 homologues detected)		This work
Rha-C12-C8	Rha-Rha-C10-C10 ^a	
Rha-C12-C10	Rha-Rha-C12-C10	
Rha-C12:1	Docenoyl-Rha-C10-C10	
Rha-C14:1	Rha-Rha-C12-C12	
Main carbon source= glycerol (8 homologues detected)		54
Rha-C10-C8	Rha-Rha-C10-C8	
Rha-C10-C10	Rha-Rha-C10-C10 ^a	
Rha-C10-C12:1	Rha-Rha-C10-C12:1	
Rha-C10-C12	Rha-Rha-C10-C12	
Main carbon source=glycerol (12 homologues detected)		55
Rha-C10-C10 ^a	Rha-Rha-C10-C10 ^a	
Rha-C8-C10	Rha-Rha-C8-C10	
Rha-C10-C8	Rha-Rha-C10-C8	
Rha-C12-C10	Rha-Rha-C12-C10	
Rha-C10-C12	Rha-Rha-C10-C12	
Rha-C10-C12:1	Rha-Rha-C10-C12:1	
Main carbon source=glycerol (10 homologues detected)		56
Rha-C10-C10	Rha-Rha-C10-C10	
Rha-C12-C10	Rha-Rha-C8-C10	
Rha-C0-C12	Rha-Rha-C10-C8	
Rha-C10	Rha-Rha-C12-C10	
	Rha-Rha-C10-C12	
	Rha-Rha-C10	

Table S4. Proteomic identification of intracellular proteins of *P. aeruginosa* strain NBTU-01. The proteins are classified according to their biological functions.

Accession No.	Score	Coverage (%)	Unique peptides	MS-MS Peptides	Avg. Mass (kDa)	Matching Protein	Source of protein (Bacterial species)	Protein family	Biological Significance
A. Key enzymes for L-rhamnose and rhamnolipid biosynthesis									
gi 310696647	31.59	6	3	3	47.08	RhIB	<i>Pseudomonas aeruginosa</i>	DEAD box helicase family	Lipid glycosylation, RNA catabolic process
gi 957651990	20.79	5	1	1	35.98	TDP-rhamnosyltransferase 2 RhIC		Glycosyltransferase family 2	Glycolipid biosynthetic process
gi 15596327	32.93	7	2	2	35.92	rhamnosyltransferase	<i>Pseudomonas aeruginosa</i> PA O1	Glucose-1-phosphate thymidyltransferase family	dTDP-rhamnose biosynthetic process, extracellular polysaccharide biosynthetic process
gi 15600356	20.97	4	1	1	32.45	glucose-1-phosphate thymidyltransferase			
gi 685843212	37.51	12	3	3	39.54	dTDP-D-glucose 4 6-dehydratase	<i>Pseudomonas aeruginosa</i>	NAD(P)-dependent epimerase/dehydratase family	dTDP-rhamnose biosynthetic process, dTDP-4-dehydrorhamnose 3,5-epimerase activity
gi 27262962	30.93	12	2	2	20.76	dTDP-4-keto-6-deoxy-D-glucose 3,5 epimerase		dTDP-4-dehydrorhamnose 3,5-epimerase family	
gi 974971520	26.72	4	1	1	32.62	dTDP-4-dehydrorhamnose reductase	<i>Pseudomonas syringae</i> pv. <i>tomato</i>	dTDP-4-dehydrorhamnose reductase family	dTDP-rhamnose biosynthetic process, extracellular polysaccharide biosynthetic process
B. Transcriptional factors involved in regulation of rhamnolipid production									
gi 68347586	85.83	29	5	7	27.54	DNA-binding response regulator AlgR	<i>Pseudomonas protegens</i> Pf-5	LytR/AlgR family	Phosphorelay signal transduction system, Transcription, Transcription regulation
gi 426270388	23.47	8	1	2	27.65	two-component			

						response regulator AlgR			
gi 359763600	23.75	10	1	2	27.61	AlgR	<i>Pseudomonas fluorescens</i> F113		
gi 15599917	42.02	7	1	1	17.34	suppressor protein DksA	<i>Pseudomonas aeruginosa</i> PA O1	DksA family	Negative regulation of lipid biosynthetic process, proteolysis, transcription and positive regulation of cellular respiration
gi 404306098	26.34	14	1	1	16.92	DksA	<i>Pseudomonas fluorescens</i> R124		
gi 10764670	25.95	5	1	2	69.4	GidA	<i>Pseudomonas syringae</i> pv. <i>syringae</i>	MnmG family	tRNA processing, tRNA wobble uridine modification, oxidoreductase activity
C. Proteins involved in other cellular functions									
gi 15599581	356.78	30	14	29	57.08	molecular chaperone GroEL	<i>Pseudomonas aeruginosa</i> PA O1	Chaperonin (HSP60) family	Protein folding
gi 999193	196.34	58	12	13	10.26	GroES	<i>Pseudomonas aeruginosa</i>	GroESchaperonin family	
gi 71557054	190.65	31	10	25	57.19	chaperonin, 60 kDa	<i>Pseudomonas savastanoi</i> pv. <i>phaseolicola</i> 1448A	Chaperonin (HSP60) family	
gi 730464226	113.08	12	5	6	77.75	elongation factor G	<i>Pseudomonas aeruginosa</i>	Classic translation factor GTPase family	Protein biosynthesis
gi 844726625	138.51	24	1	11	43.38	elongation factor Tu			
gi 576715139	177.51	30	6	14	43.52	translation elongation factor Tu	<i>Pseudomonas</i> sp. GM30		
gi 15599473	227.01	39	14	22	43.37	elongation factor Tu	<i>Pseudomonas aeruginosa</i> PA O1		
gi 489255485	47.44	2	1	1	90.75	translation initiation factor IF-2	<i>Pseudomonas aeruginosa</i>		

gi 643985067	24.3	2	1	1	74.96	methionine-- tRNA ligase		Class-I aminoacyl- tRNAsynthetase family	
gi 518316414	92.13	5	2	3	77.58	elongation factor G		Classic translation factor GTPase family	
gi 565827107	42.88	2	1	1	50.11	dihydrolipoyl dehydrogen ase			Glycolysis
gi 320329264	108.84	9	5	5	50.10	dihydrolipoa mide dehydrogen ase	<i>Pseudomonas savastanoipv. glycinea str. race 4</i>	Class-I pyridine nucleotide-disulfide oxidoreductase family	Glycolysis, Oxidoreductase activity, cell redox heomstasis
gi 852183060	116.2	14	6	6	50.16	dihydrolipoyl dehydrogen ase	<i>Pseudomonas aeruginosa</i>		Glycolysis
gi 730736148	82.56	9	3	3	39.44	phosphoglyc erate kinase		Phosphoglycerate kinase family	
gi 15596035	35.41	9	1	1	17.26	glutathione peroxidase	<i>Pseudomonas aeruginosaPA O1</i>	Glutathione peroxidase family	
gi 759433819	41.56	2	1	1	88.66	endopeptida se La	<i>Pseudomonas aeruginosa</i>	Peptidase S16 family	
gi 15599956	64.28	12	2	2	20.70	heat shock protein GrpE	<i>Pseudomonas aeruginosaPA O1</i>	GrpE family	
gi 15599955	222.98	35	15	21	68.40	molecular chaperone DnaK		Heat shock protein 70 family	
gi 60549563	123	10	1	7	68.74	DnaK	<i>Pseudomonas putida</i>		Stress response
gi 685935753	123.09	8	6	7	103.41	Clp protease ClpC		ClpA/ClpB family	
gi 743510175	103.89	16	1	7	68.38	molecular chaperone DnaK		Heat shock protein 70 family	
gi 730495440	86.44	9	5	5	71.66	molecular chaperone HtpG	<i>Pseudomonas aeruginosa</i>	Heat shock protein 90 family	
gi 959920977	103.05	26	5	5	15.71	universal stress protein UspA		Universal stress protein A family	

gi 685940847	71.93	3	2	2	50.29	serine protease MucD		Peptidase S1C family	
gi 959888827	64.47	22	4	4	17.28	lipid hydroperoxide peroxidase		Peroxiredoxin (PRX) family	
gi 959864041	59.15	3	1	2	91.62	ATP-dependent chaperone ClpB		ClpA/ClpB family	
gi 489181310	48.14	15	2	2	20.42	MULTISPECIES: tellurium resistance protein TerE	<i>Pseudomonas</i>	terD/terE/terZ family	
gi 1001954107	40.82	9	1	1	16.52	UspA domain-containing protein	<i>Pseudomonas aeruginosa</i>	Universal stress protein A family	
gi 759371605	74.49	11	1	1	14.70	peroxiredoxin OsmC		OsmC/Ohr family	
gi 685895501	64.95	9	2	2	31.83	chaperone protein HchA		Peptidase C56 family	
gi 404302499	32.92	15	2	2	20.89	GrpE	<i>Pseudomonas fluorescens</i> R124	GrpE family	
gi 730411503	43.65	5	1	1	19.87	alkylhydroperoxidase	<i>Pseudomonas aeruginosa</i>	AhpD family	
gi 730441130	28.88	7	1	1	16.21	alkylhydroperoxidase			
gi 928214863	28.98	2	1	1	65.58	acyl-CoA dehydrogenase	<i>Pseudomonas fuscovaginae</i>	Acyl-CoA dehydrogenase family	Oxidoreductase activity, acyl coA dehydrogenase activity
gi 499793935	21.41	2	1	1	48.48	cytochrome P450	<i>Rhodopseudomonas palustris</i>	Cytochrome P450 family	Monooxygenase activity
gi 32967107	26.59	4	2	2	58.91	LapN	<i>Pseudomonas alkylphenolia</i>	Methane/Phenol/Toluene Hydroxylase family	Cellular aromatic compound metabolic process

gi 489230774	20.6	3	1	1	40.13	muconatecy cloisomerase		Mandelateracemas e/muconatelactonizing enzyme family	Amino acid catabolism, aromatic hydrocarbon catabolism	
gi 490472260	110.66	22	5	5	42.95	dihydrolipoidesuccinyltransferase	<i>Pseudomonas aeruginosa</i>	2-oxoacid dehydrogenase family	TCA Cycle	
gi 686194421	126.65	17	4	4	45.40	malate dehydrogenase		MDH type 2 family		
gi 928214574	27.54	3	2	2	79.78	isocitrate dehydrogenase, NADP-dependent, monomeric type	<i>Pseudomonas fuscovaginae</i>	Isocitrate dehydrogenase family		
gi 576714049	121.88	25	8	8	29.94	succinyl-CoA synthetase, alpha subunit	<i>Pseudomonas sp.</i> GM30	Succinate/malate CoA ligase beta subunit family		
gi 15599529	44.46	4	3	3	54.76	fumarase	<i>Pseudomonas aeruginosa</i> PAO1	Class-II fumarase/aspartase family		
gi 913661954	24.63	2	1	1	63.67	succinate dehydrogenase	<i>Pseudomonas fluorescens</i> NCIMB 11764	FAD-dependent oxidoreductase 2 family		
gi 759852432	79.34	8	3	3	41.53	succinyl-CoA ligase subunit beta	<i>Pseudomonas aeruginosa</i>	Succinate/malate CoA ligase beta subunit family		
gi 564009570	44.98	3	1	1	40.36	citrate synthase/methylcitrate synthase		Citrate synthase family		
gi 557557463	67.35	8	5	5	66.11	pyruvate carboxylase	<i>Pseudomonas aeruginosa</i> VR FPA05	Pyruvate carboxylase family		Biotin carboxylase activity, gluconeogenesis
gi 15596597	26.74	2	2	2	116.88	pyruvate carboxylase	<i>Pseudomonas aeruginosa</i> PAO1			
gi 331024675	21.55	3	1	1	53.95	acetyl-CoA hydrolase	<i>Pseudomonas coronafaciens pv. oryzae str.</i>	Acetyl-CoA hydrolase/transferase family	Acetyl CoA metabolic process	

							1_6			
gi 666690932	25.92	2	1	1	72.72	acetyl-CoA hydrolase	<i>Pseudomonas amygdalipv. tabaci str. 6605</i>			
gi 15600131	29.54	6	2	2	46.81	adenylosuccinatesynthetase	<i>Pseudomonas aeruginosa</i> PAO1	Adenylosuccinatesynthetase family	Nucleotide biosynthesis	
gi 730562452	137.75	32	5	5	15.59	nucleosidediphosphate kinase	<i>Pseudomonas aeruginosa</i>	NDK family		
gi 730628152	83.53	32	3	3	14.27	nucleosidediphosphate kinase regulator, partial				
gi 15598882	21.51	4	1	1	23.11	adenylate kinase	<i>Pseudomonas aeruginosa</i> PAO1	Adenylate kinase family		
gi 489208621	41.8	3	1	1	38.31	dihydroorotase	<i>Pseudomonas aeruginosa</i>	DHOase family		
gi 759362708	45.09	11	1	1	13.01	5-hydroxyisourate hydrolase		Transthyretin family		
gi 730604841	32.57	3	1	1	36.13	adenosine deaminase		Adenosine and AMP deaminases family		
gi 100093550_1	26	2	1	1	63.28	hemolysinactivation/secretion protein		ShIB/FhaC/HecBfamily		Protein transport
gi 835861098	109.46	10	4	4	48.46	molecular chaperone SurA	<i>Pseudomonas aeruginosa</i>	Rotamase family		Protein transport, protein stabilization
gi 640489896	88.33	19	3	3	11.73	phospholipid-binding protein		BON family		Phospholipid transport
gi 553789555	106.93	15	6	6	39.80	branched chain amino acid ABC transporter substrate-		Bacterial solute-binding protein 3 family	Transport activity	

						binding protein		
gi 959920221	58.33	23	2	2	13.01	azurin		Plastocyanin/azurin family
gi 1001954259	104.02	15	3	3	33.07	ABC transporter		Bacterial solute-binding protein 3 family
gi 15600433	134.12	42	4	4	11.87	thioredoxin	<i>Pseudomonas aeruginosa</i> PA O1	Thioredoxin family
gi 959898859	114.85	24	5	5	32.19	ABC transporter substrate-binding protein		Solute-binding family 1
gi 685987587	88.11	15	3	3	23.79	toluene tolerance protein		MlaC/ttg2D family
gi 685900046	86.76	16	4	4	34.53	porin		OmpA family
gi 510933646	52.51	5	2	2	59.73	ABC transporter substrate-binding protein		Solute-binding family 1
gi 553799256	42.57	6	1	1	23.10	outer membrane lipoprotein carrier protein LolA	<i>Pseudomonas aeruginosa</i>	LolA family
gi 959875038	38.8	8	2	2	26.45	electron transporter RnfB		4Fe4S bacterial-type ferredoxin family
gi 553794897	182.82	28	2	13	45.16	sugar ABC transporter substrate-binding protein		Binding-protein-dependent transport system permease family
gi 751334327	130.54	13	4	4	39.83	branched chain amino acid ABC transporter substrate-		Bacterial solute-binding protein 3 family

						binding protein			
gi 959862992	41.57	4	1	1	29.78	amino acid ABC transporter substrate-binding protein		Bacterial solute-binding protein 3 family	
gi 730657412	36.15	4	1	1	26.51	ABC transporter substrate-binding protein		Solute-binding family 1	
gi 959922431	35.07	6	1	1	20.70	ubiquinol-cytochrome c reductase iron-sulfur subunit		Oxidoreductase family	
gi 518201378	25.66	1	1	1	127.44	sensor histidine kinase		Solute carrier 5 family	
gi 489199932	32.6	3	1	1	38.24	ABC transporter substrate-binding protein		Bacterial solute-binding protein 3 family	
gi 489252733	32.04	4	1	1	30.42	phosphonate ABC transporter substrate-binding protein			
gi 489239673	32.55	2	1	1	46.48	porin		OprB family	
gi 787858374	24.68	4	2	2	55.37	F0F1 ATP synthase subunit alpha	<i>Pseudomonas chlororaphis</i>	ATPase alpha/beta chains family	ATP synthesis, transport activity
gi 787858376	62.26	9	3	3	49.44	F0F1 ATP synthase subunit beta			
gi 489190867	40.14	4	2	2	55.38	ATP synthase	<i>Pseudomonas aeruginosa</i>		

						subunit alpha				
gi 685898620	90.21	13	4	4	40.50	spermidine/putrescine ABC transporter substrate-binding protein	<i>Pseudomonas syringae</i> pv. <i>tomato</i> T1	Binding-protein-dependent transport system permease family	Polyamine transport	
gi 730561914	68.78	4	1	1	37.05	C4-dicarboxylate ABC transporter		Tripartite ATP-independent periplasmic transporter family	Periplasmic transporter activity	
gi 553756307	96.99	12	3	3	36.53	sulfate ABC transporter substrate-binding protein		Prokaryotic sulfate-binding protein family	Secondary active sulfate transmembrane transporter activity, sulfate transmembrane-transporting ATPase activity, sulphur compound metabolic process	
gi 553777858	93.4	3	2	2	55.58	catalase		Catalase family	Hydrogen peroxide catabolic process, response to hydrogen peroxide	
gi 213928538	38.16	3	2	2	82.66	catalase/peroxidase HPI		Peroxidase family		
gi 730239109	92.05	5	3	3	115.39	ribonuclease E		<i>Pseudomonas aeruginosa</i>	RNase E/G family	RNA catabolic process, tRNA and rRNA processing
gi 959948237	91.59	17	3	3	23.37	isoprenoid biosynthesis protein ElbB			Not specified	Transferase activity
gi 489254440	81.63	2	3	3	178.73	Large exoproteins involved in heme utilization or adhesion			ShIA/HecA/FhaA family	Cell adhesion
gi 1001043234	81.06	10	2	2	34.50	phosphate-binding protein	PstS family		Cell adhesion, Phosphate transport, Transport, Virulence	

gi 742352091	78.87	11	2	2	15.94	copper chaperone		CopZ family	Chaperone, copper ion transport
gi 730686201	28.24	9	1	1	13.06	50S ribosomal protein L19		Ribosomal protein L19P family	Translation
gi 950499024	78.28	13	2	2	22.62	50S ribosomal protein L3		Ribosomal protein L3P family	
gi 1001046479	74.07	17	2	2	21.96	50S ribosomal protein L25/general stress protein Ctc		Ribosomal protein L25P family	
gi 15599935	57.25	24	1	1	10.12	30S ribosomal protein S15		Ribosomal protein S15P family	Translation regulation
gi 730383240	37.64	6	1	1	24.21	50S ribosomal protein L1		Ribosomal protein L1P family	
gi 730526514	48.02	1	2	2	150.04	DNA-directed RNA polymerase subunit beta		RNA polymerase beta chain family	Transcription
gi 514414261	43.35	1	1	1	154.10	DNA-directed RNA polymerase subunit beta			
gi 730609375	29.51	4	1	1	25.62	DNA-binding response regulator		Not specified	Transcription regulation
gi 730522340	45.61	10	1	1	12.80	transcriptional regulator		Not specified	
gi 822019811	86.58	12	3	3	34.35	RNA polymerase sigma-H factor AlgU		Sigma-70 factor family	
gi 15595653	60.33	19	1	1	7.71	cold-shock protein	<i>Pseudomonas aeruginosa</i> PAO1	CSD family	

gi 988890275	55.46	13	1	1	13.37	transcriptional regulator	<i>Pseudomonas aeruginosa</i>	Not specified	
gi 100104551 2	39.99	7	1	1	20.18	transcription termination/ antitermination protein NusG		NusG family	
gi 730278173	46.34	6	2	2	40.68	DNA polymerase III subunit beta		DNA polymerase type-C family	Replication
gi 759434958	55.7	22	2	2	9.70	DNA-binding protein HU-alpha		Bacterial histone-like protein family	DNA Condensation
gi 15597001	71.86	27	3	3	9.09	DNA-binding protein HU	<i>Pseudomonas aeruginosa</i> PAO1		
gi 822018644	70.18	12	2	2	13.69	rod shape-determining protein RodA	<i>Pseudomonas aeruginosa</i>	SEDS family	Cell cycle, cell division, regulation in cell shape
gi 510945409	69.65	5	3	3	45.42	glutamate-1-semialdehyde 2,1-aminomutase		Class-III pyridoxal-phosphate-dependent aminotransferase family	Porphyrin biosynthesis
gi 489210699	69.32	20	4	4	20.54	peroxiredoxin		AhpC/TSA family	Peroxiredoxin activity, cell redox homeostasis, Antioxidant, Oxidoreductase
gi 100105577 7	67.85	12	1	1	16.44	6,7-dimethyl-8-ribityllumazine synthase		DMRL synthase family	Riboflavin biosynthesis
gi 685899032	123.75	35	6	6	15.33	homoserine acetyltransferase		HTA family	Amino acid biosynthesis
gi 759862093	56.98	1	1	1	117.32	carbamoyl phosphate synthase large subunit		CarB family	

gi 740618957	59.94	7	2	2	28.11	4-hydroxy-tetrahydrodipicolinate synthase		DapA family	
gi 553786862	60.2	6	1	1	36.49	ketol-acid reductoisomerase		Ketol-acid reductoisomerase family	
gi 730406310	62.66	7	2	2	34.12	branched chain amino acid aminotransferase		Class-IV pyridoxal-phosphate-dependent aminotransferase family	
gi 749659485	86.08	9	5	5	44.16	aspartate kinase		Aspartokinase family	
gi 333113900	40.7	12	4	4	51.86	glutamate synthase, small subunit	<i>Pseudomonas fulva</i> 12-X	Glutamate synthase family	
gi 768690557	26.99	1	2	2	161.59	putative glutamate synthase [NADPH], large subunit	<i>Pseudomonas sp.</i> HMSC05H02		
gi 489213558	43.25	4	1	1	28.50	tryptophan synthase subunit alpha	<i>Pseudomonas aeruginosa</i>	TrpA family	
gi 489249800	38.8	4	1	1	49.10	cystathionine beta-synthase		Cystathionine beta-synthase family	
gi 565826207	39.36	6	2	2	51.96	glutamine synthetase		Glutamine synthetase family	
gi 489253047	30.74	3	1	1	43.71	tryptophan synthase subunit beta		TrpB family	
gi 582004101	48.14	14	3	4	48.82	glutamate dehydrogenase	<i>Pseudomonas pseudoalcaligenes</i> CECT 5344	Glu/Leu/Phe/Val dehydrogenases family	Amino acid metabolism
gi 15599784	25.46	7	1	2	48.86	glutamate dehydrogenase	<i>Pseudomonas aeruginosa</i> PA O1		

gi 928212666	64.03	18	6	6	43.33	acetylornithine/succinylornithine aminotransferase	<i>Pseudomonas fuscovaginae</i>	Class-III pyridoxal-phosphate-dependent aminotransferase family	
gi 727810341	28.63	3	1	1	44.53	N-succinylglutamate 5-semialdehyde dehydrogenase	<i>Pseudomonas aeruginosa</i>	Aldehyde dehydrogenase family	
gi 6002915	26.75	5	2	2	39.77	chloromuconate cycloisomerase		Mandelate racemase/muconate lactonizing enzyme family	Amino acid catabolism
gi 1000934201	32.34	4	1	1	38.67	L-asparaginase		Asparaginase 1 family	
gi 730718422	61.52	15	1	3	28.22	methionine ABC transporter substrate-binding protein		DNA mismatch repair MutS family	Mismatch repair
gi 730551508	81.81	22	2	4	28.10	methionine ABC transporter substrate-binding protein			
gi 759865090	58.84	13	2	2	17.28	ecotin		Protease inhibitor I11 (ecotin) family	Protease inhibitor
gi 751334232	29.13	7	1	1	17.32	ecotin			
gi 959838988	58.3	21	2	2	17.51	chemotaxis protein CheW		CheW family	Signal transducer activity, chemotaxis
gi 499198003	54.83	1	1	1	92.98	nitrate reductase catalytic subunit		Prokaryotic molybdopterin-containing oxidoreductase family	Electron transport, nitrate assimilation, transport, oxidoreductase activity
gi 518225896	54.7	3	1	1	59.76	gamma-glutamyltran		Gamma-glutamyltransferase	Glutathione biosynthesis

						speptidase		family	
gi 320330409	27.26	8	1	1	32.00	thioredoxin	<i>Pseudomonas savastanoipv. glycinea str. race 4</i>	Thioredoxin family	Cell redox homeostasis, glycerol ether metabolic process,
gi 553762809	52.42	8	2	2	31.94	co-chaperone YbbN	<i>Pseudomonas aeruginosa</i>	ybbN protein family	Oxidoreductase activity
gi 490480111	50.44	5	2	2	25.31	succinyl-CoA--3-ketoacid-CoA transferase		3-oxoacid CoA-transferase family	Ketone body catabolic process
gi 501655356	45.37	10	2	2	23.98	ketohydroxy glutarate aldolase		KDPG/KHG aldolase family	2-dehydro-3-deoxy-phosphogluconate aldolase activity, 4-hydroxy-2-oxoglutarate aldolase activity
gi 489188245	40.72	4	1	1	33.82	MULTISPECIES: thioredoxin-disulfide reductase		<i>Pseudomonas</i>	Class-II pyridine nucleotide-disulfide oxidoreductase family
gi 730332388	44.32	8	1	1	23.41	thiol:disulfide interchange protein DsbA	<i>Pseudomonas aeruginosa</i>	Thioredoxin family	Cell redox homeostasis, protein folding
gi 553752507	41.01	7	1	1	12.92	PA-I galactophilic lectin		PA-IL-like protein family	Carbohydrate binding
gi 489255221	39.24	3	1	1	41.95	chitin-binding protein CbpD		CBP family	Carbohydrate metabolic process
gi 558688971	40.57	19	2	2	13.35	4a-hydroxytetrahydrobiopterin dehydratase		Pterin-4-alpha-carbinolamine dehydratase family	Tetrahydrobiopterin biosynthetic process
gi 852075221	40.35	3	2	2	73.71	PrkA family serine		PrkA family	Protein kinase activity

						protein kinase			
gi 759456745	39.3	24	1	1	9.68	chromosome partitioning protein ParA		ParA family	Chromosome segregation
gi 489194016	28.76	2	1	1	64.35	MULTISPECIES: acyl-CoA dehydrogenase	<i>Pseudomonas</i>	Acyl-CoA dehydrogenase family	Oxidoreductase activity
gi 959882353	29.4	2	1	1	41.78	acyl-CoA dehydrogenase			
gi 730279592	36.89	6	1	1	22.19	nitroreductase		Nitroreductase family	
gi 685899885	35.78	4	1	1	35.69	amino acid dehydrogenase		Oxidoreductase family	
gi 553763722	26.36	2	1	1	38.63	oxidoreductase			
gi 565900676	53.21	5	2	2	50.07	aldehyde dehydrogenase		Aldehyde dehydrogenase family	
gi 501025821	82.76	8	3	3	53.15	aldehyde dehydrogenase	<i>Pseudomonas aeruginosa</i>		
gi 501025052	82.5	18	2	2	21.37	superoxide dismutase [Fe]		Iron/manganese superoxide dismutase family	
gi 1256708	32.4	12	2	2	29.14	dihydrodiol dehydrogenase		Short-chain dehydrogenases/reductases (SDR) family	
gi 730284259	24.83	2	1	1	35.19	NADPH:quinone reductase		Medium chain Dehydrogenases/reductase (MDR)/zinc-dependent alcohol dehydrogenase-like family	
gi 145692874	24.63	6	2	2	36.33	HCH-reductase		Oxidoreductase family	

						LinF		
gi 100106831 2	34.14	9	1	1	17.44	Hcp1 family type VI secretion system effector		Hcp1 family Endonuclease activity
gi 730333325	33.68	2	1	1	53.56	biofilm formation protein PslB		Mannose-6- phosphate isomerase type 2 family Polysaccharide biosynthetic process
gi 730304091	33.52	8	1	1	13.21	two- component system response regulator		Not specified Two-component regulatory system, transcription regulation
gi 640474228	32.71	11	1	1	16.71	nitrogen regulatory protein		Not specified Phosphoenolpyruvate- dependent sugar phosphotransferase system, kinase activity
gi 852081788	26.52	5	1	1	20.67	pilus assembly protein PilZ		PilZ family Pilus assembly
gi 730301699	26.26	3	1	1	41.57	acetyl-CoA acetyltransfe rase		Thiolase family Lipid metabolism
gi 15598209	21.55	3	1	1	41.64	3-ketoacyl- CoA thiolase	<i>Pseudomonas aeruginosa</i> PA O1	
gi 989001359	47.28	10	1	1	16.43	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	<i>Pseudomonas aeruginosa</i>	AccD/PCCB family Lipid biosynthesis
gi 553798646	23.43	8	1	1	12.56	DNA-binding transcription al activator OsmE		SmpA / OmlA family DNA binding
gi 953533548	23.42	4	1	1	31.69	metallopepti dase		Zn peptidase family Hydrolase activity, metalloprotease activity

gi 553765499	23.18	1	1	1	70.99	protease		Trypsin 2/ Endonuclease NS family	Protease activity
gi 959908322	22.92	3	1	1	33.98	type III secretion system protein		FliMN C family	Cell motility, Intracellular trafficking, secretion, and vesicular transport
gi 730357728	22.32	8	1	1	12.18	iron donor protein CyaY		FrataxinCyaY family	Oxidative stress response, cellular iron ion homeostasis, glutathione metabolic process
gi 959913462	53.04	15	1	1	11.19	peptidase		Peptidase family	Hydrolase activity
gi 730265471	75.57	7	2	2	47.25	peptidase			
gi 730520115	30.46	6	1	1	19.42	inorganic pyrophosph atase		PPase family	Hydrolase activity, phosphate-containing compound metabolic process
gi 489176416	21.69	2	1	1	40.18	MULTISPE CIES: peptidase M14	<i>Pseudomonas</i>	Succinylglutamated esuccinylase / Aspartoacylase family	Hydrolase activity
gi 759368125	54.61	15	2	2	16.38	histidine triad (HIT) protein		Histidine triad (HIT) protein family	
gi 640476987	21.47	1	1	1	141.14	type VI secretion system protein ImpL		IcmF family	Intracellular trafficking, secretion, and vesicular transport
gi 730721957	21.36	3	1	1	36.49	DNA topoisomera se IV subunit B	<i>Pseudomonas aeruginosa</i>	Type II topoisomerase family	Antibiotic resistance, Chromosome organization
gi 935268401	22.17	1	1	1	94.27	hypothetical protein			
gi 959888333	27.58	15	1	1	7.65	hypothetical protein			
gi 489206432	21.13	1	1	1	72.51	hypothetical protein, partial		Unknown	Unknown activity
gi 959841889	21.15	7	1	1	26.89	hypothetical protein			

gi 640477118	46.5	5	1	1	22.46	hypothetical protein		
gi 917622807	47.96	15	1	1	9.14	hypothetical protein		
gi 968123518	59.6	21	2	2	13.33	hypothetical protein		
gi 100105840 4	63.24	14	3	3	21.35	hypothetical protein		
gi 727810136	76.13	40	1	1	63.65	hypothetical protein, partial		
gi 763409557	162.98	22	8	8	51.25	hypothetical protein		
gi 685886565	81.58	21	2	2	18.29	membrane protein		

Table S5. The alignments of MS-MS derived peptide sequences of *P. aeruginosa* strain NBTU-01 with the rhamnolipid synthesizing enzymes reported in the databases. The peptides identified by MS/MS sequencing in this proteome using PEAKS 7.0 software are shown in bold and putative conserved domains have been underlined.

(1) Accession No.: gi|310696647

Matching Protein: RhIB

Bacterial Species: *Pseudomonas aeruginosa*

MHAILIAIGSAGDVFPFIGLARTLKLGRHRSVSLCTIPVFRDAVEQHGIAFVPLSDELTYRRTMGDPRLWDPKTSFGVLWQTIAGMIEPVYEVSAQRHD
DIVVVGSLWALGARIAHEKYGIPYLSAQVSPSTLLSAHLPPVHPK**FNVPEQMPLAMR**KLLWRCIERFK**LDRTCAPDINAVRR**KVGLLETPVKRIFTQWM
HSPQGVVCLFPAWFAPPQQDWPQPLHMTGFPLFDGSIPTLDELQRFQDQSRPLVFTQGSTEHLQGDYFAMALRALERLGARGIFLTGAGQE
PLRGLPNHVLQRAYAPLGLPSCAGLVHPGGIGAMSLALAAGVPQVLLPCAHDQFDNAERLVRLGCGMRLGVPLREQELRGALWRLLLEDPAMAA
ACRRFMELSQPHSIACGKAAQVVERCHREGDARWLKAAS

(2) Accession No.: gi|957651990

Matching Protein: TDP-rhamnosyltransferase 2 RhIC

Bacterial Species: *Pseudomonas aeruginosa*

MDRIDMGVLVWLFNPGDDDD**LEHLGELSAAFPQLR**FLAVDNSPHSDPQRNARLRGQGIADVLYHGNRQGIAGAFNQGLDTLFRRGLQGVLLLDQDSR
PGGAFLAAQWRNLQACNGQACLLGPRIFDRGDRRFLPAIHLDGLALRQLSLDGLTTPQRTSFLISSGCLLTREAYQRLGHFDEELFIDHVDTEYSLRA
QALDVPLYVDPRLVLEHRIGTRKTRRLGGLSLSAMNHAPLRRYYLARNGLLVLRYYARSSPLALLANLPTLTQGLAVLLLERDKLLKLRCLGWGLWD
GLRGRGGALERNRPRLLKRLAGPAVASVASGKAKA

(3) Accession No.: gi|15596327

Matching Protein: rhamnosyltransferase

Bacterial Species: *Pseudomonas aeruginosa PAO1*

MDRIDMGVLVWLFNPGDDDD**LEHLGELAAAFPQLRFLAVDNSPHSDPQR**NARLRGQGIADVLYHGNRQGIAGAFNQGLDALFRRGVQGVLLLDQDSR
PGGAFLAAQWRNLQAR**NGQACLLGPR**IFDRGDRRFLPAIHLDGLTLRQLSLDGLTTPQRTSFLISSGCLLTREAYQRLGHFDEELFIDHVDTEYSLRA
QALDVPLYVDPRLVLEHRIGTRKTRRLGGLSLSAMNHAPLRRYYLARNGLLVLRYYARSSPLALLANLPTLTQGLAVLLLERDKLLKLRCLGWGLWD
GLRGRGGALETNRPRLLKRLAGPAVASVASGKAKA

(4) Accession No.: gi|15600356

Matching Protein: glucose-1-phosphate thymidyltransferase

Bacterial Species: *Pseudomonas aeruginosa PAO1*

Putative conserved domain: Glyco_tranf_GTA_type superfamily

MKRKGIILAGGSGTRLHPATLAISKQLLPVYDKPMIYYPLSTLMLAGIREILIIISTPQDTPRFQQLLGDGSNWGLDLQYAVQPSPDGLAQAFDIGESFIG
NDLSALVLDGDNLYYGHDFHELLGSASQRQTGASVFAYHVLDPERYGVVEFDQGGKAISLEEKPLEPKSNYAVTGLYFYDQQVVDIARDLKPSPRGEL
EITDVNRAYLERGQLSVEIMGRGYAWLDTGTHDSLLEAGQFIATLENRQGLKVACPEEIA YRQKWIDAAQLEKLAAPLAKNGYGQYLKRLLTETVY

(5) Accession No.:gi|685843212

Matching Protein:dTDP-D-glucose 4 6-dehydratase

Bacterial Species:*Pseudomonas aeruginosa*

MTILVTGSAGFIGANFVLDWLALHDEPVVSLDKLTYAGNRQNLASLDGDARHTFVAGDIGDSQLVARLLAEHQPRAILNFAAESHVDRSIHGPEDFIQ
TNIVGTFRLLEEVRRAYWGALEPEAKAAFRFLHVSTDEVYGS LAPSDPAFTENNRYPNSPYSASKAASDHLV RAYHHTYGLPVLTTNCSNNYGPYH
FPEKLIPLVIHNALAGKPLPIYGDGQQIRDWLYVKDHCSAIRRVLEAGQLGETYNVGGWNEKANLDVVETLCAILDQEQPRADGRSYREQITFVKDRP
GHDRRYAIDATRLERELGWKPAETFETGIRKTVRWYLDNQDWVANVTSGAYREWVGKQYA

(6)Accession No.:gi|27262962

Matching Protein:dTDP-4-keto-6-deoxy-D-glucose 3,5 epimerase

Bacterial Species:*Pseudomonas aeruginosa*

MKATRLAIPDVILFEPRVFGDDRGFFESYNQRAFEEACGHPVSFVQDNHSRSARGVLRGLHYQIRQAQGKLV RATLGEVFDVAVDLRRGSPTFGQ
WVGERLSAENKRQMWIPAGFAHGFFVVLSEYAEFLYKTTDFWAPEHERCIVWNDPELKIDWPLQDAPLLSEKDRQGKAFADADCFP

(7)Accession No.: gi|974971520

Matching Protein: dTDP-4-dehydrorhamnose reductase

Bacterial Species: *Pseudomonas syringaepv. Tomato*

MRMRLMLLGGGNALGQALIRLGAENIGFLAPRPPQDGWDAASLTQLLDDTRPDALINLAYYFDWFQAESVSEARLTHQERSVERLAELCQHNNITL
VQPSSYRVFDGSRATAYSEKDEPVPLGLRGQALWRIEQSVRATCPQHVLVRFVWLLDSDADGVLGRFLSRAKEPGELLLADDRRGNPTPVDDAAR
VIISVIKQLDCEAPLWGTYHYAGHEATTPLALGQAVLTEARLLHPLAIESPTAQAHAARPDAAEPPQHAVLACKKILHTFGIKPRAWRAGLPSLLDRYY
RHV

Table S6. Surface tension measurement of crude and TLC purified rhamnolipid biosurfactant produced by *P. aeruginosa* strain NBTU-01 at different pH. Values are mean \pm S.D. of three determinations.

pH	Surface Tension (mNm ⁻¹)	
	Crude rhamnolipid	Purified dirhamnolipid
3.0	72.9 \pm 0.4	70.9 \pm 0.2
4.0	70.9 \pm 1.1	69.9 \pm 0.3
5.0	68.1 \pm 0.9	63.1 \pm 0.8
6.0	30.6 \pm 1.0*	29.5 \pm 0.5*
7.0	30.8 \pm 0.5*	31.8 \pm 0.3*
8.0	31.7 \pm 1.2*	32.7 \pm 0.6*
9.0	38.2 \pm 2.1*	33.2 \pm 0.3*
10.0	58.9 \pm 0.1**	55.9 \pm 0.1**
11.0	71.8 \pm 0.2	70.8 \pm 0.2
12.0	71.2 \pm 0.9	70.7 \pm 0.3

Surface tension of the control (deionized water) was 72.2 \pm 0.6 mN/m,
 Statistical analysis: *p < 0.001, ** p < 0.05 with respect to control value.

Table S7. Effect of heating at 100 °C for different time interval on surface tension reduction property of crude and purified rhamnolipid biosurfactant produced by the *P. aeruginosa* strain NBTU-01. Values are mean \pm S.D. of three determinations.

Duration of Heating at 100 °C (min)	Surface tension (mNm ⁻¹)	
	Crude rhamnolipid	Purified dirhamnolipid
5	31.5 \pm 0.29*	29.5 \pm 0.22*
10	31.6 \pm 0.46*	29.7 \pm 0.46*
20	31.7 \pm 0.65*	29.7 \pm 0.65*
30	30.6. \pm 1.0*	29.8 \pm 1.0*
60	31.7 \pm 0.66*	30.7 \pm 0.66*
120	32.7 \pm 0.22*	31.7 \pm 0.22*
240	32.2 \pm 0.39*	31.2 \pm 0.39*
300	31.9 \pm 0.69*	30.9 \pm 0.69*

Surface tension of the control (deionized water) was 72.2 \pm 0.6 mN/m,

Statistical analysis: *p < 0.001 with respect to control value.