Supplementary data

Lipopeptide biosurfactant from *Bacillus clausii* BS02 using sunflower oil soapstock: Evaluation of high throughput screening methods, production, purification, characterization and its insecticidal activity

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Enrichment, isolation and selection of biosurfactant producers

Each sample (soil, 5.0 g; water, 5.0 ml; approximately) was added to a 500 ml Erlenmeyer flask containing 100 ml of pre-sterilized Basal salt medium (BSM) [containing (g l^{-1}) $(NH_4)_2SO_4$, 0.9; KH_2PO_4, 0.7; K_2HPO_4, 1.5; $MgCl_2$, 0.5, $MgSO_4$, 0.1 and 10 ml of trace element solution containing (g l^{-1}): $ZnSO_4.7\ H_2O$, 2.32; $MnSO_4.4\ H_2O$, 1.78; $CuSO_4.5\ H_2O$, 1.0; EDTA, 1.0; KCl, 0.66; H_3BO_3, 0.56; $CoCl_2.6\ H_2O$, 0.42; $Na_2MoO_4.2\ H_2O$, 0.39; $NiCl_2.6\ H_2O$, 0.004; pH 7.0±0.2] broth to which 2% v/v diesel or crude glycerol was added as the sole carbon source. After 3 days of incubation in a rotary shaker (Steelmet Industries, Pune, India) at 120 rpm, 5 ml of culture broth was sampled from each flask and transferred into a second batch of flasks containing fresh medium; these flasks were then incubated under the same conditions to decrease the unwanted microbial load. This process was repeated thrice, and each time 5 ml of culture broth was withdrawn from the 'older' flasks and transferred into new ones. After enrichment, aliquots (100 µl) of serial dilutions (10^{-8}) of the culture broth from the last batch of flasks were spread on agar plate with the same composition of the enrichment medium. The resulting plates were incubated at ambient temperature under aerobic conditions for 1-2 days, until colony formation. The morphologically different bacterial colonies which developed on the plates were streaked on nutrient agar plates to obtain pure cultures of the isolates, and were maintained on nutrient agar slants and kept at 4^{o}C in the refrigerator.

Contact angle measurements

A Standard Goniometer (Model no. 200-F4, Rame´-Hart, Netcong, NJ) was used for this purpose. A drop of cell-free culture supernatant was placed on a cleaned surface (1×1×2 cm^3) at room temperature, and contact angle was determined using the Rame´-Hart Imaging 2001 software package. The volume of the sessile drop was maintained as 5 µl in all cases using a microsyringe. The contact angle was measured within 45-60 s of the addition of the liquid

drop with an accuracy of ±1°. Measurements were repeated at least five times to check the accuracy. Also contact angles were measured with definite time intervals for a single drop and the measurements were recorded as snap shots. A fluid is said to wet a solid surface if the contact angle is < 90^{o} (for surfactant containing liquids). If the contact angle is > 90^{o}, the fluid is said to be non-wetting.[1,2]

Identification of the selected isolate

Preliminary phenotypic and biochemical characterization

Phenotypic characterization of the shortlisted isolates was done based on their colony morphology, microscopic observations, and biochemical tests. For this purpose, BSM broth or agar was used as the basal medium. Cell morphology was observed using a light microscope (model: BX51, OLYMPUS, Japan) and field emission scanning electron microscope (FESEM, model: S-4800, Hitachi, Japan) after 24 h growth on BSM agar at 30^{o}C. Morphological properties were studied according to general protocols[1] following growth on BSM agar after 48 h at 30^{o}C. The Gram reaction was determined by the conventional Gram staining method by using the Gram staining kit (HiMedia, Mumbai, India). Spore staining were done using standard protocol.[3] Cell motility was determined with an optical microscope using the hanging drop method.[4]

Physiological tests such as growth at different temperatures, pH and tolerance to NaCl were examined on BSM medium at 30^{o}C, unless otherwise indicated. Biochemical characterization which included oxidase and catalase reaction, nitrate reduction, IMViC tests, urease tests, phenylalanine deaminase activity, acid or gas production from carbohydrates etc. were performed using KB002 HiAssorted^{TM} Biochemical test kit according to the manufacturer's instructions (HiMedia, Mumbai, India) for the isolate BS01. All of the tests

were performed in duplicate. The isolates were identified identified according to Bergey's Manual of Determinative Bacteriology.[5]

Whole-cell fatty acid Methyl Ester (FAME) profiling

This analysis was performed at Royal Life Sciences Pvt. Ltd., Hyderabad (affiliated to MIDI Sherlock, USA). Pure culture of the isolates were cultivated on Trypticase Soy Broth Agar (TSBA) plates at 28^{o}C for 24 h. Cellular fatty acid methyl esters of freshly grown culture were obtained by a four step method (saponification, methylation, extraction and washing) as per MIDI manual (Microbial Identification, Inc.). The samples were injected in to a Gas Chromatograph (model: 6850 Series II, Agilent, USA) equipped with a flame ionization detector and 30 m Rtx®-5 (fused silica) capillary column (Restek, Bellefonte, PA). Ultra-high purity hydrogen was used as a carrier gas and column head pressure was 60 kPa. Injector and detector temperatures were 300^{o}C and 240^{o}C, respectively. Temperature of oven was programmed to increase from 170^{o}C to 270^{o}C at a rate of 5^{o}C min^{-1}. Fatty acid profiles were identified with Sherlock software version 6.0B (RTSBA6 library version 6.00, MIDI). The peaks were automatically named and quantitated by the system. A similarity (SIM) index cutoff of 0.6 was used to determine confident species match, unless otherwise specified. Qualitative and quantitative differences in the fatty acid profiles were used to compute the distance for each strain relative to the strains in the library.

16S rDNA sequencing and phylogenetic analysis

The taxonomic characterization on the basis of nucleotide sequence of 16S rDNA was confirmed through an external agency (Royal Life Science Pvt. Ltd., Hyderabad, India). DNA extraction of a single colony was performed as per Ausubel *et al*.[6] PCR amplification of the 16S rRNA gene was performed using universal primer16sF (5'-AGA GTT TGA TCC TGG CTC AG-3') and 16sR (5'-ACG GCT ACC TTG TTA CGA CTT-3') on a GeneAMP

PCR System 9700 thermal cycler in 25 µl reaction mixture. The PCR conditions comprised of (i) initial denaturation step (95°C, 10 min), (ii) 25 cycles of (a) denaturation (95°C, 1 min), (b) annealing (55°C, 1 min) (c) extension (72°C, 1.30 min) and (iii) final extension (72°C, 10 min). PCR products were purified using Qiagen PCR Product Purification kit before subjecting to fluorescence-based ABI BigDyeTM terminator chemistry as per manufacturer's instructions. DNA cycle sequencing was performed in automated ABI 3730XL DNA Analyzer (Applied Biosystems, USA) and basecalled using Sequence Scanner v1.0. Sequence similarities were inferred from NCBI GenBank database using BLAST[7] for phylogenetic analysis using sequences of the related taxa. Neighbor joining tree[8] was constructed based on distance matrices calculated according to the Kimura two-parameter model[9] using MEGA v5.04.[10]

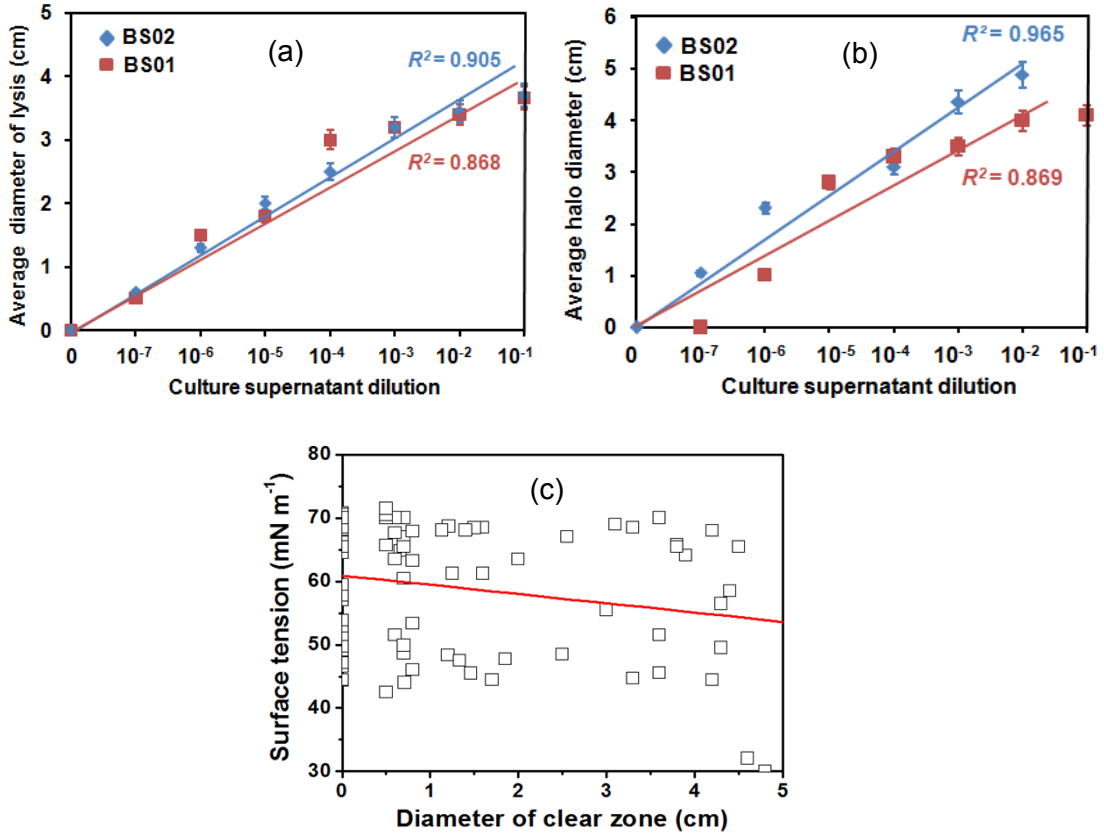

Fig. S1. The relationship between (a) the diameter of blood agar lysis (in cm) and the concentration of biosurfactant in cell-free culture broth; (b) the diameter of the clear zone obtained by the oil spreading technique (in cm) and the concentration of biosurfactant in cell-free culture broth; and (c) the diameter of the clear zone obtained by the oil spreading

technique (in cm) and surface tension of the culture (in mN m^{-1}), where each point represents a different isolate. The solid line is the least square fit. Error bars indicate the standard deviation of three independent measurements. The solid line is the least square fit. Where the error bars are not visible, the standard deviation was within the area occupied by the symbol.

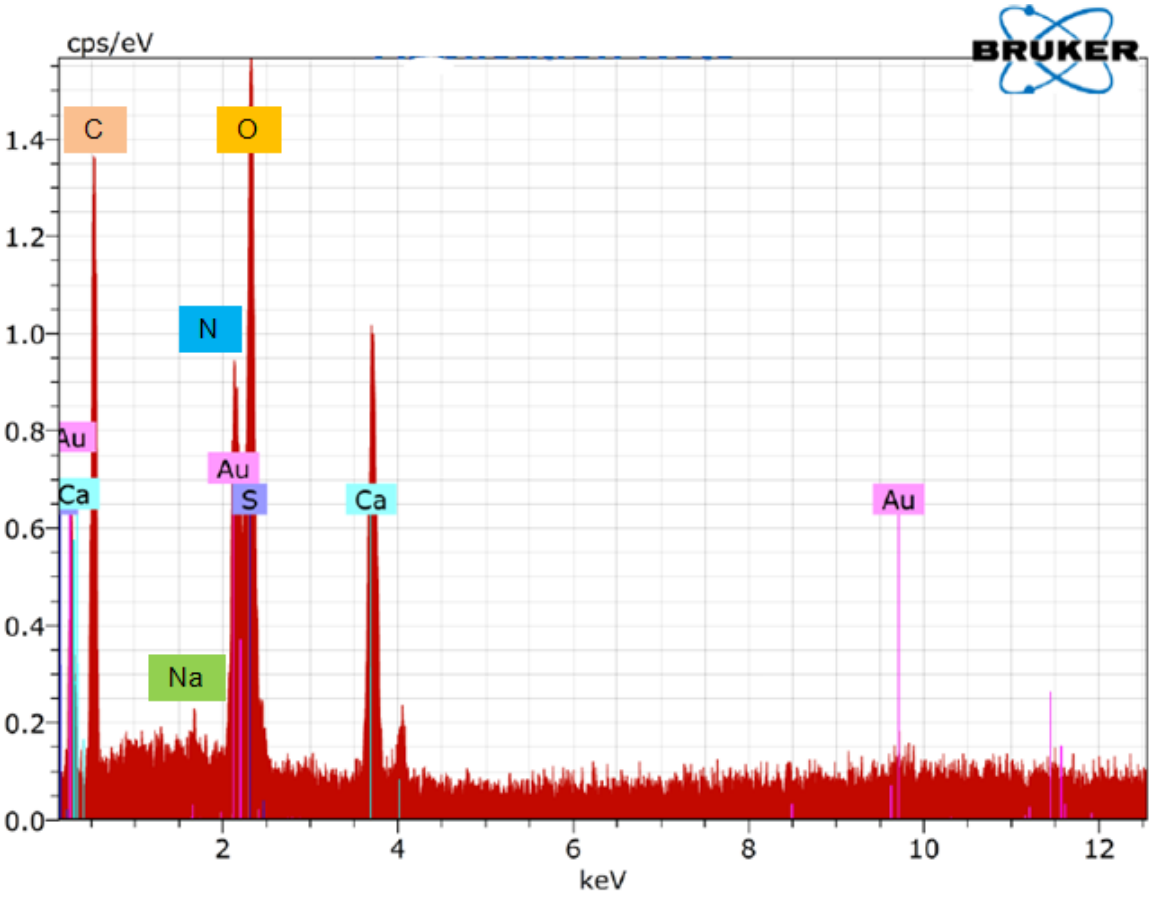

Fig. S2. EDX spectrum of the recovered crude biosurfactant obtained from *B. clausii* BS02

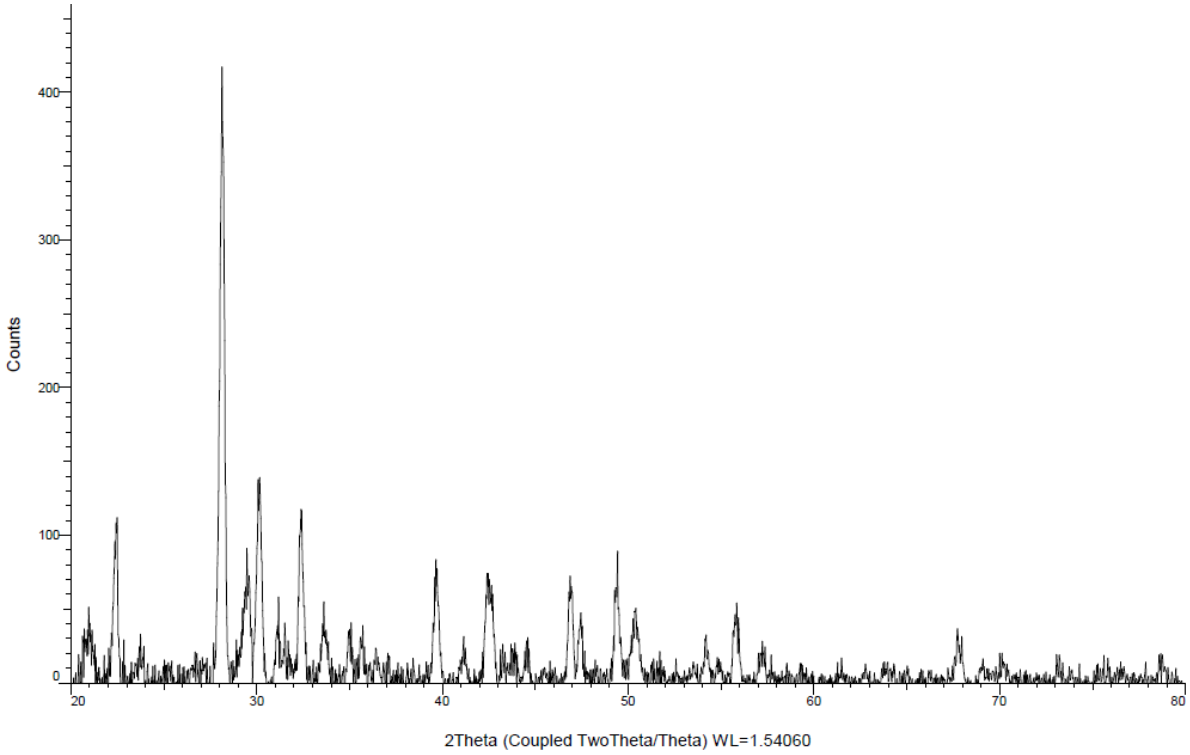

Fig. S3. Powder X-ray diffraction of the recovered crude biosurfactant obtained from *B. clausii* BS02

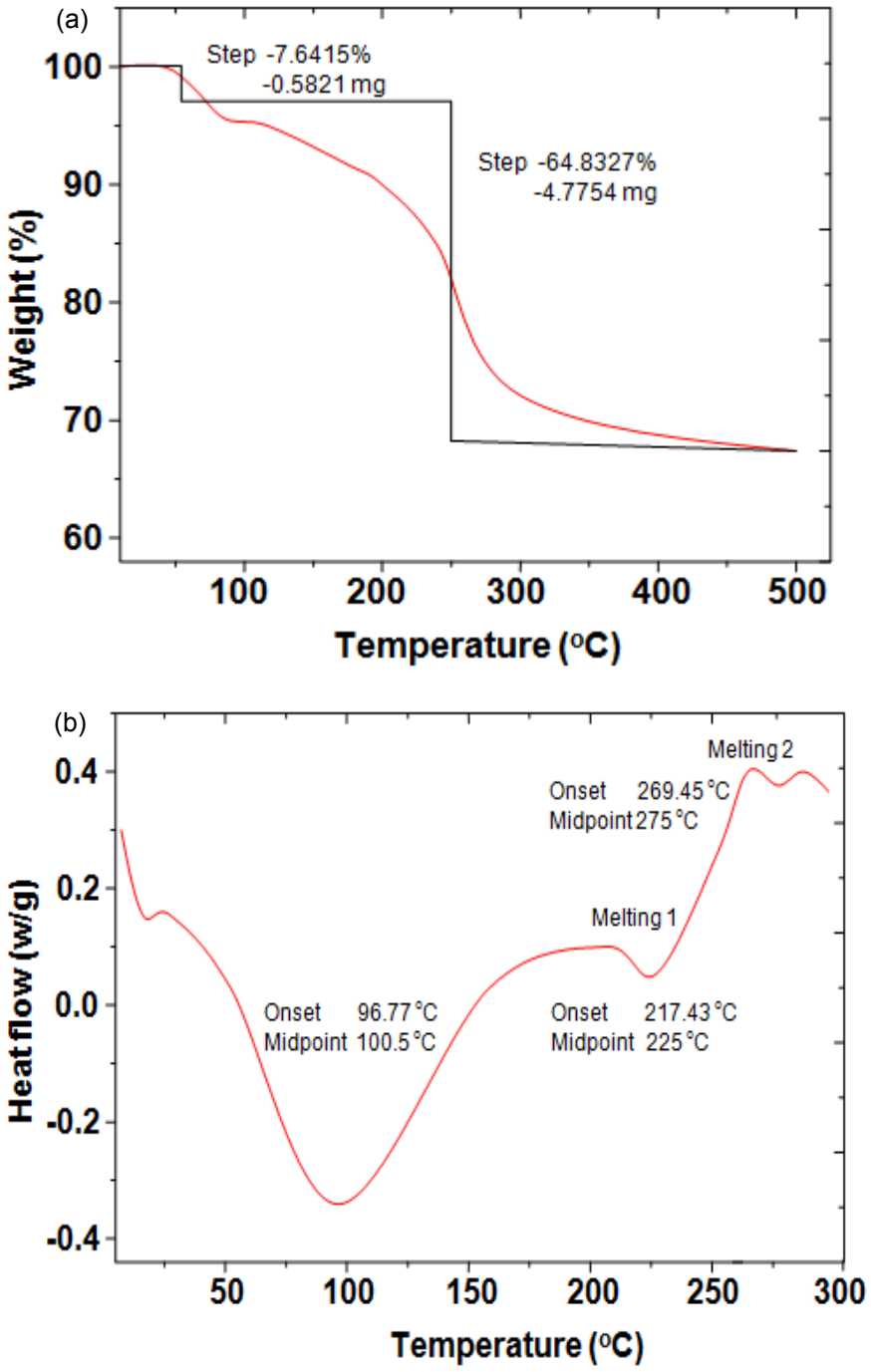

Fig. S4. (a) TG and (b) DSC thermogram of crude biosurfactant obtained from *B. clausii* BS02 at heating rate of 10°C.

Table S1. Details of the sampling sites and their relative bacterial composition

Sampling site	Site description	No. of total isolates	
		Gram-negative	Gram-positive
Soil			
Petrol pump station	Soil mixed with petrol and diesel	**18**	**04**
Garage installations	Receives vehicle run-off	**05**	**01**
Hydrocarbon contaminated area	Receives run-off from environment	**05**	**02**
Pesticide contaminated region	Prevalent history of pesticide contamination and collects sippage from nearby solvent producing industry	**04**	**01**
Artificially created kerosene contaminated locale	Residential garden soil, maintained with daily dose of kerosene for a month	**04**	**07**
Sewage effluent			
Coconut wastewater	Receives effluent from public street vendor	**05**	**05**
Dairy wastewater	Water canal by milk factory and dairy farm	**03**	**07**
Oily sludge			
Temple	Receives edible oils	**02**	**0**
Mustard oil production facility	Waste oily sludge from production unit	**02**	**01**

Table S2. Assessment of various screening methods for detecting biosurfactant activity among tested bacterial isolates (contd. on next page)

Isolate	Assay methods															
	1	2	3	4		5	6	7	8	9	10	11	12	13	14	15	16
				K	D												
Gram positive																	
BS02	+	+	++	88.91	81.57	++++	+	+	+	++	+	+	+++	-	32.07	75°	+++
BS03	+	+	+	18.92	17.65	+	-	-	-	+	-	-	-	-	48.4	88°	+
BS04	+	+	++	22.5	25	+++	-	+	+	+	-	+	++	-	44.5	83°	-
BS06	+	+	+	2.7	57.14	+	-	+	+	+	-	+	++	-	65.7	99°	++
BS16	+	+	+	10.81	38.46	++	-	+	+	++	-	+	+++	-	68.7	105°	+
BS19	+	+	+	16.66	64.71	-	-	+	+	-	-	+	+	-	66.05	101°	+
BS20	-	-	++	41.66	46.67	-	-	+	+	-	-	+	+	-	46.5	82°	++
BS21	-	-	+	31.57	62.5	-	-	+	+	-	-	+	+	+	44.3	81°	+
BS22	-	-	+	68.42	50	-	-	+	+	-	-	+	+++	-	48.08	85.5°	-
BS23	+	+	-	5.26	12.5	+++	+	+	+	+	+	+	+	-	47.5	83°	-
BS24	+	+	+	74.35	80	+	-	-	-	++	-	-	-	-	44.03	82°	-
BS25	+	+	+	67.56	20	++	-	+	+	-	-	+	+++	-	45.5	84°	+
BS26	+	-	-	61.72	32	-	-	+	+	+	+	-	-	+	49.04	88°	-
BS27	+	+	-	63.15	23.53	+	-	+	+	-	-	-	+	-	51.5	89°	+
BS28	+	+	+	51.3	31.2	+++	-	+	+	-	-	-	+++	-	64.08	101°	-
BS29	+	+	++	31.5	43.7	-	-	+	+	+	-	-	-	-	70.5	109°	-
BS30	+	+	-	84.2	35.7	-	-	-	-	-	-	-	-	-	70.8	108°	-
BS33	+	+	-	72.9	42.8	-	-	-	-	+	-	-	-	-	70.6	106°	-
BS34	+	+	-	47.2	68.7	-	-	+	+	-	-	-	+	-	69.5	104°	-
BS35	+	+	+	54	68.7	++	-	+	+	+	-	-	-	-	68.07	105°	-
BS36	+	-	+	59.4	50	-	-	+	+	-	-	-	-	-	52.55	92°	+
BS37	+	-	++	81	20	-	-	+	+	-	-	-	-	-	51.07	95.5°	+
BS38	-	-	-	2.6	20	-	-	+	+	-	-	-	+++	-	49.05	93.5°	-
BS39	+	+	+	70.2	76.9	++	+	+	+	+	+	+	+	-	61.27	103°	+
BS40	+	+	-	48.3	68.7	+	-	+	+	-	-	-	+	+	64.84	106°	-
BS45	+	+	+	65.5	56.2	+++	-	+	+	+	-	-	+++	-	65.77	107°	+
BS47	+	-	+	44.8	64.7	-	-	+	+	-	-	-	-	-	69.03	108°	+
BS48	+	-	-	60	70.5	-	-	+	+	-	-	-	-	-	68.5	102°	-
BS49	+	+	+	69.3	76.9	-	-	+	+	-	-	-	-	-	68.08	102°	-
BS51	+	+	-	59.3	64.2	+	-	+	+	-	-	-	+	-	70.04	101°	-
BS52	+	-	-	67.8	58.8	-	-	+	+	+	-	-	-	-	64.45	86.7°	-
BS53	+	-	+	70	52.9	-	-	+	+	-	-	-	-	-	68.19	104°	-
BS54	+	+	++	63.3	53.8	+++	-	+	+	-	-	-	-	-	67.05	105°	-
BS55	+	+	+	77.7	66.6	++++	-	+	+	-	-	-	+	-	68.03	103°	-
BS56	+	+	+	66.6	62.5	+	-	+	+	-	-	-	+	-	67.08	102°	-
BS57	+	+	+	73	58.8	++++	-	+	+	+	-	-	+	-	65.5	105°	+
BS58	+	+	+	66.6	16.6	++	-	+	+	-	-	-	++	+	63.27	102°	+
BS59	+	+	++	61.2	50	+	-	+	+	-	-	-	+	-	49.5	94°	+
BS61	+	+	-	66.6	62.5	+	-	+	+	-	-	-	-	+	48.6	93°	-
BS63	+	+	+	60	73.3	++	-	+	+	+	-	-	-	+	44.5	91°	-
BS64	+	+	-	58	85.7	+	-	+	+	-	-	-	-	-	68.5	103°	-

Table S2. contd

Isolate	Assay methods															
	1	2	3	4		5	6	7	8	9	10	11	12	13	14	15	16
				K	D												
Gram negative																	
BS01	+	+	++	92.77	91.65	++++	++	-	-	++	+	+	+++	+	30.06	76°	+++
BS05	+	+	+	5	84.62	++	-	+	+	++	+	+	+	-	47.8	88°	+
BS07	+	+	+	25	73.33	-	+	-	-	+	+	+	+	-	44.5	83°	+
BS08	+	+	+	27.5	70.59	++++	+	+	+	+	+	+	+++	+	56.5	102°	+
BS09	+	+	-	2.56	68.75	+	+	+	-	+	-	-	+	+	63.5	107°	+
BS10	+	+	-	24.3	20	-	-	+	+	+	-	-	-	-	68.8	108°	-
BS11	+	+	+	21.4	11.76	+	-	+	+	+	-	-	+++	+	53.4	89.8°	-
BS12	+	+	-	40.5	66.67	++++	+	+	+	-	-	-	+	-	58.5	88.5°	-
BS13	-	-	++	10.8	18.75	-	-	+	+	+	-	-	-	-	57.08	94°	-
BS14	+	+	+	10.5	8.33	-	-	+	+	++	-	-	+++	-	49.7	96.4°	-
BS15	+	+	+	10.5	8.33	++	++	+	+	-	-	-	-	-	65.5	104°	-
BS17	+	+	++	44.4	38.46	-	+	+	+	+	-	-	-	-	66.16	106°	-
BS18	+	+	-	80	26.67	+	++	+	+	++	-	-	-	-	70.04	101°	-
BS31	+	+	-	87.8	20	-	-	-	-	-	-	-	-	-	53.83	91°	-
BS32	+	+	-	71.4	46.15	++++	-	-	-	-	-	-	-	-	51.5	87.2°	-
BS41	+	+	+	66.5	83.33	+	+	+	+	+	-	-	-	-	70.05	106°	-
BS42	+	+	-	60	66.67	++	-	+	+	+	-	-	-	-	68.44	103°	-
BS43	+	+	+	11.1	53.3	+	-	+	+	+	-	-	++	-	67.9	102°	-
BS44	+	+	+	72.4	45.4	++	-	+	+	+	-	-	-	+	61.25	87.5°	-
BS46	+	+	+	46.6	64.2	-	-	+	+	+	-	-	-	-	57.81	89°	-
BS50	+	+	+	60	61.5	-	-	+	+	+	-	-	+	+	59.5	89.5°	-
BS60	+	+	-	53.3	78.3	+	++	-	-	-	-	-	-	-	60.45	101°	+
BS62	+	+	-	51.7	33.3	++++	-	+	+	+	-	+	-	-	65.5	90.5°	+
BS65	+	+	-	63.6	78.5	+	-	+	+	-	+	-	+	+	67.63	92°	+
BS66	+	+	-	60	66.6	-	++	+	+	+	+	-	-	-	70.06	91.7°	-
BS67	+	+	+	60	66.67	+	-	+	+	++	-	-	-	-	70.05	107°	-
BS68	+	+	+	11.1	53.3	+	-	+	+	+	-	-	++	-	70.5	105°	-
BS69	+	+	+	72.4	45.4	+++	-	+	+	+	-	-	-	+	68.55	101°	-
BS70	+	+	+	46.6	64.2	+	-	+	+	++	-	-	-	-	49.88	92°	-
BS71	+	+	-	60	61.5	-	-	+	+	+	-	-	+++	+	47.17	96° 98°	-
BS72	+	+	+	53.3	78.3	+	+	-	-	-	-	-	-	-	46.05	101°	-
BS73	+	+	-	51.7	33.3	-	-	+	+	+	-	+	+	-	68.5	103°	-
BS74	+	+	-	63.6	78.5	+++	-	+	+	+	+	-	-	+	69.03	104°	-
BS75	+	+	-	60	66.6	+	-	+	+	+	+	-	-	-	71.5	86.4°	-
BS76	+	+	+	60	64.2	-	-	+	+	-	-	-	+++	-	70.04		+

Key: 1, tilted glass slide test; 2, Parafilm M test; 3, drop collapse test; 4, EI_{24} assay, K: kerosene and D: diesel; 5, oil spreading assay; 6, CTAB-MB assay; 7, droplet assay; 8, penetration assay; 9, microplate assay; 10, replica plate method; 11, HOA assay; 12, blood agar hemolysis, and 13, BATH assay; 14, surface tension; 15, water contact angle measurement; 16, atomized oil assay.

For blood agar hemolysis: '-', no hemolysis; '+', hemolysis with a clear zone of 1-1.5 cm; '++', complete hemolysis with a diameter of lysis 1.6-2.5 cm; '+++', complete hemolysis with a diameter of lysis 2.6-4 cm. For the oil spreading technique: '+' average halo diameter between 0.5 and 0.9 cm, '++' average halo diameter between 1 and 1.5 cm, '+++' average halo diameter >1.5 and < 3 cm, and '++++' average halo diameter >3. 1 and < 5 cm. For atomized oil assay: '-', no halo; '+' average halo radius between 1 and 2 mm, '++' average halo radius between 2 and 4 mm, and '+++' average halo radius > 4 and < 9 mm. For other assays: '-' negative and '+' positive reaction.

Table S3. Comparative analysis of methods for detection of biosurfactant production (n= 76)

Sr. no.	Method	Positive by method			Negative by method		
		No. of positives	No. (%) of strains with identical response	Response level	No. of negatives	No. (%) of negatives that were positive with other methods	Method number
1.	Blood agar haemolysis	41	13 (31%) 06 (07%) 22 (53%)	+++[a] ++ +	35	23 (66%) 12 (34%)	2 and 3 3
2.	Drop collapse	54	09 (17%) 45 (83%)	++ +	22	14 (63%) 08 (36%)	3 4 and 5
3.	Oil spreading	51	08 (16%) 07 (14%) 12 (24%)	++++[b] +++ ++	25	09 (36%) 16 (64%)	4 1
4.	CTAB-MB	16	06 (37%) 10 (62%)	++ +	60	36 (60%) 24 (40%)	3 and 5 2
5.	Microplate	48	09 (19%) 39 (82%)	++ +	28	17 (60%) 11 (39%)	1 4

[a] Hemolysis assay: +, incomplete hemolysis; ++, complete hemolysis with a diameter of lysis < 1 cm; +++, complete hemolysis with a diameter of lysis < 1 cm but < 3 cm and ++++, complete hemolysis with a diameter of lysis > 3 cm and green colonies. [b] Oil spreading assay: +, diameter of clearing zone between 0.5 and 0.9 cm; ++, diameter of clearing zone between 1 and 1.5 cm; +++, diameter of clearing zone between > 1.5 and < 2.1 cm and ++++, diameter of clearing zone > 2.1 and < 3 cm. n, number of isolates tested.

Table S4. Statistical correlations between different methods

	Spearman rank correlation coefficient (r_s)					Test of independence correlation coefficient ($_2$)				
	Blood agar	**Drop collapse**	**Oil spreading**	**CTAB-MB**	**Microplate**	**Blood agar**	**Drop collapse**	**Oil spreading**	**CTAB-MB**	**Microplate**
Blood agar	1	0.541	0.453	0.258	0.316	1	0.286	0.213	0.202	0.185
Drop collapse		1	0.91	0.335	0.284		1	0.82	0.157	0.193
Oil spreading			1	0.569	0.455			1	0.271	0.254
CTAB-MB				1	0.358				1	0.22
Microplate					1					1

Table S5. Biochemical and physiological properties of the isolate BS02

Characteristics	BS02
Morphology on BSM agar amended with diesel/crude glycerol (2%, v/v)	
Gram Stain	+
Colony shape	circular
Colony size (mm)	0.7-1.0
Cell shape/morphology	rod
Cell size (µm) (length × width)	0.8-1.0×2.0-2.5
Elevation	slightly raised/low convex
Edge/Margin	rhizoid
Surface	shiny and smooth
Opacity	translucent
Motility	motile
Spore formation	+
Pigmentation on:	
NB	-
LB	-
BSM (+diesel/glycerol)	-
Physiological tests	
Growth at temperature (°C)	
04	-
41	+
Optimum growth temperature (°C)	30
pH range for growth	6.5-10.0
pH optima	7.0-8.0
NaCl range for growth (%, w/v)	1-10
IMViC	
Indole production	-
Methyl red test	-
Voges Proskauer test	-
Citrate utilization	-
Reduction of nitrate to nitrite	+
H_2S production	-
Utilization of:	
D-Glucose	+/w
D-Maltose	+
D-Mannitol	w
L-Rhamnose	-
Sucrose	+
Fructose	+
Trehalose	-
Xylose	+
Enzyme profile	
α-Amylase	-/w
Argenine dihydrolase	w
Catalase	+

Characteristics	BS02
Gelatinase	+
Oxidase	+
Urease	-
Hydrolysis of:	
Tween 20	-
Tween 80	-
Starch	w
Casein	+
Gelatin	+

+, positive/growth; -, negative/no growth; w, weak growth

Table S6. Relative abundance and profiling of cellular fatty acids of the isolate BS02

Fatty acid[a]	Profile (%)	Fatty acid[a]	Profile (%)
$C_{9:0}$	-	anteiso-$C_{17:0}$	5.8
$C_{10:0}$	-	$C_{16:0}$ 3OH	-
$C_{10:0}$ 3OH	-	$C_{16:1}$ *ω5c*	-
$C_{11:0}$	-	$C_{16:1}$ *ω7c*	-
iso-$C_{11:0}$ 3OH	-	$C_{16:1}$ *ω9c*	-
$C_{11:0}$ 3OH	-	$C_{16:1}$ *ω11c*	3.7
$C_{12:0}$	-	$C_{16:1}$ *ω*7c alcohol	4.4
$C_{12:0}$ 2OH	-	iso-$C_{17:0}$	9.5
$C_{12:0}$ 3OH	-	iso-$C_{17:0}$ 3OH	2.2
$C_{13:0}$	-	iso-$C_{17:1}$ *ω10c*	1.8
iso-$C_{13:0}$	-	$C_{17:0}$ cyclo	-
$C_{14:0}$	-	$C_{17:0}$	1.0
iso-$C_{14:0}$	3.4	$C_{17:1}$ *ω8c*	-
$C_{14:1}$ *ω7c*	0.7	$C_{18:0}$	1.3
$C_{14:1}$ *ω5c*	-	$C_{18:1}$ *cis* 11	0.5
$C_{15:0}$	-	$C_{18:1}$ *ω9c*	1.5
iso-$C_{15:0}$	38.5	$C_{18:1}$ *ω7c*	1.0
anteiso-$C_{15:0}$	28.4	$C_{19:0}$ cyclo	-
$C_{15:1}$ *ω8c*	-	$C_{19:0}$ cyclo *ω8c*	-
$C_{15:1}$ *ω6c*	-	Summed Feature*	
$C_{16:1}$ *trans* 9	-	1	-
$C_{16:1}$ *cis* 9	-	3	1.6[b]
$C_{16:0}$	-	5	-
iso-$C_{16:0}$	3.5	8	12.4[c]

[a]*ω*, Methyl end of fatty acid, *c*, cis configuration of the double bond, cyclo, cyclopropane fatty acid, iso, branched fatty acids, OH indicates the position of hydroxyl group from the acid end. Fatty acids representing less than 0.1% in all strains were omitted. Summed features represent two or three fatty acids that cannot be separated by GLC using the MIDI system.*Summed features represent two or three fatty acids that cannot be separated by GLC using the MIDI system [b]$C_{16:1}$ *ω6c* /$C_{16:1}$ *ω7c*, [c]$C_{18:1}$ *ω*7c/$C_{18:1}$ *ω*6c.

Table S7. Effect of various concentration of sunflower soapstock on growth, surface tension, emulsification activity and biosurfactant production by *B. clausii* BS02.

Soapstock (%, w/v)	**ST (mN m^{-1})[a]**	**EI_{24}% [a]**	**Biomass (g l^{-1})[a]**	**Biosurfactant (g l^{-1})[a]**
0.5	31.7±0.4	72±0.3	1.2±0.2	1.2±0.01
1	31.2±0.2	76±0.4	1.3±0.1	1.5±0.04
2	30.5±0.3	78±0.1	1.4±0.1	1.7±0.02
3	30	82±0.4	1.6±0.2	2.6±0.03
4	30.5±0.1	77±0.3	1.5±0.3	2.2±0.04
5	30.5±0.3	75±0.1	1.4±0.2	2.0±0. 1

[a] The data are mean ± standard deviation of three independent experiments.

Table S8: Literature reports on the production of lipopeptide biosurfactants using various economical substrates.

Strain	Carbon source (g l^{-1})	Biosurfactant (g l^{-1})	Reference(s)
Bacillus subtilis C9	Glucose (40)	13.5	11
B. subtilis ATCC 21332	Mineral salt+glucose (40)	3.5	12
B. subtilis LB5a	Cassava wastewater	3.0	13
B. subtilis #573	Mineral salt+sucrose	2.15	14
B. subtilis PT2	Nutrient broth+plam oil (20-80)	-	15
Bacillus I-15	Glucose (10 mM)	0.2	16
B. subtilis EG1	Luria-Bertani medium	0.15	17
Sphingobacterium detergens	MCA medium+glucose+n-alkane	0.46	18
B. pumilis KS3	Mineral medium+glucose (20)	2.37	19
B. methyloptrophicus USTBa	MSM medium+crude oil (20)	1.8	20
B. subtilis LSFM-05	BSM+biodiesel glycerol (50)	1.37	21
B. circulans	Glycerol mineral salts medium	2.9	22
B. circulans	Starch mineral salts medium	2.5	22
B. subtilis SPB1	Tuna fish flour (4.34 g)+ potatowaste flour (5.66 g)	27.1 mg g^{-1}	23
Bacillus	Basal medium containing goat tallow	2.03 g g^{-1} of goat tallow	24
B. subtilis DM-03	M9 media and 2.0% (w/v) potato peel	80.0 mg gds^{-1}	25
B. amyloliquefaciens XZ-173	Soybean flour+rice straw+maltose (20 w/w)+ glycerol (26.5 w/w)	15.03 mg gds^{-1}	26
B. subtilis strains CCTCC M201162	Beer wastewater (40)	1.26	27
B. circulans	Glucose mineral salts (20)	1.0	28
B. licheniformis TKU004	Squid pen powder (10)	0.55	29
B. subtilis ATCC 21332	Landy medium	0.2	30
B. subtilis ATCC 21332	Landy medium	0.2-0.8	31
B. subtilis B20	Date molasses (80)	2.29	32

B. licheniformis	Bushnell-Haas medium	10.0	33
B. pumilus UFPEDA 448	Okara (500)+sugarcane bagasse (500)	0.80	34
B. subtilis LAMI005	Clarified cashew apple juice (total reducing sugar content: 20)	0.35	35
B. subtilis LAMI005	Glycerol (20)	0.44	36
B. clausii isolate BS02	BSM medium + sunflower soapstock (30)	2.5	This study

Table S9: Physicochemical and biochemical properties of the crude biosurfactant of *B. clausii* BS02 cultivated on BSM medium supplied with sunflower soapstock (3%, w/v).

Properties	**Values**
Physical appearance	White semisolid
Biochemical analysis (% composition)	
(a) Protein	67.3±2.5
(b) Lipid	28.0±2.1
(c) Carbohydrate	4.5±2.8
Surface tension reduction (mN m^{-1})	30.0±0.4
Interfacial tension against n-hexadecane (mN m^{-1})	1.5±0.2
CMC[a] (mg l^{-1})	45±0.4
Emulsification activity (A_{600} nm) against	
(a) Benzene	0.636
(b) Kerosene	0.885
(c) Paraffin	0.753
(d) n-hexadecane	0.857
Percent activity remains after heating the crude biosurfactants[b]	
(a) Surface tension reduction	94.0±0.5
(b) CMC	100.0
SR[c] for oil	23.55×10^{-2}
SR for fat	76.18×10^{-2}
Foam height[d]	77%

Results are mean±S.D. of three independent experiments; [a]CMC, critical micelle concentration; [b]At 100°C for 60 min; [c]SR, solubilization ratio; [d]Calculated after vigorous shaking of the crude biosurfactant solution for 2 min using the equation: foaming = height of foam/total height × 100.

Table S10: Dose-dependent mortalities of adzuki bean weevil, *C. chinensis* (L.) and mealybug, *M. hirsutus* after treatment with crude biosurfactant of *B. clausii* BS02 by vapor toxicity bioassay.

Treatment dose (µg ml^{-1})	**Mortality (%)***			
	12 h	**24 h**	**36 h**	**48 h**
Callosobruchus chinensis				
Control	2.76±1.52^{a}	2.85±1.55^{a}	3.68±1.51^{a}	4.17±1.55^{a}
0.1	8.77±5.37^{ab}	9.75±4.31^{ab}	20.71±4.39^{ab}	27.33±4.33^{ab}
0.5	6.85±1.64^{a}	8.89±2.61^{a}	22.18±2.66^{a}	34.81±2.58^{a}
1.0	3.81±1.41^{ab}	7.84±1.55^{ab}	17.88±1.35^{ab}	35.74±1.22^{ab}
5.0	6.73±4.33^{ab}	11.75±4.39^{ab}	23.63±4.37^{ab}	44.77±3.35^{ab}
10.0	14.12±5.57^{b}	19.48±5.31^{b}	29.44±5.39^{b}	45.71±5.51^{b}
20.0	26.35±6.28^{c}	34.37±6.28^{c}	38.31±5.22^{c}	47.33±7.69^{b}
30.0	33.74±8.25^{c}	39.72±8.28^{c}	44.78±7.21^{c}	48.75±5.18^{b}
40.0	40.55±5.58^{c}	46.64±5.59^{d}	48.62±6.55^{c}	49.62±8.19^{b}
50.0	56.15±8.23^{d}	58.13±8.51^{d}	59.11±5.52^{d}	60.11±6.21^{c}
100.0	99.64±3.88^{e}	99.55±2.84^{e}	100.0±0^{e}	100.0±0^{e}
Maconellicoccus hirsutus				
Control	1.75±1.22^{a}	2.88±1.51^{a}	3.77±1.55^{a}	4.55±1.51^{a}
0.1	8.71±4.56^{ab}	9.33±4.38^{ab}	22.70±4.39^{ab}	26.31±4.35^{ab}
0.5	8.82±1.66^{a}	8.83±2.36^{a}	24.11±2.66^{a}	38.86±2.43^{a}
1.0	5.16±1.85^{ab}	7.55±1.51^{ab}	16.48±1.32^{ab}	31.75±1.73^{ab}
5.0	7.78±5.31^{ab}	12.74±6.38^{ab}	24.11±5.33^{ab}	45.43±3.32^{ab}
10.0	15.19±5.55^{b}	18.43±4.30^{b}	30.52±5.58^{b}	41.70±6.53^{b}
20.0	25.11±7.33^{c}	36.32±8.22^{c}	39.34±5.82^{c}	46.44±3.88^{b}
30.0	30.77±8.21^{c}	38.55±6.29^{c}	45.75±5.25^{c}	47.18±3.92^{b}
40.0	42.48±6.11^{c}	45.65±4.66^{d}	47.19±6.87^{c}	48.61±5.11^{b}
50.0	55.18±7.22^{d}	59.11±6.55^{d}	59.68±4.52^{d}	61.55±4.22^{c}
100.0	95.11±2.81^{e}	97.59±2.88^{e}	100.0±0^{e}	100.0±0^{e}

*Means ± SD of triplicate. The data with the same letter in the same column are not significantly different (p <0.05, Tukey's test). Methanol was used as a solvent control.

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