**Supplementary Data**

**Analysis of Biosurfactants Produced by Bacteria Growing on Textile Sludge and Their Toxicity Evaluation for Environmental Application**

**Tables**

Table S1 Biochemical characterization and various carbon source utilization by biosurfactant producing isolates (*Stenotrophomonas* sp. BAB-6435 and *Brevisbacillus brevis* BAB-6437).

|  |  |  |  |
| --- | --- | --- | --- |
| **Test** | **Result** | **Test** | **Result** |
| **BAB-6435** | **BAB-6435** | **BAB-6435** | **BAB-6435** |
| Gram character | -ve | +ve | citrate utilization | +ve | +ve |
| Shape | rod shape | rod shape | urea hydrolysis | -ve | -ve |
| Motility | Non-motile | Motile | gelatine hydrolysis | +ve | +ve |
| Catalase activity | +ve | +ve | **Carbon source utilization** |
| Growth temp. (oC) | 25-40 | 30-55 | β-Lactose | +ve | +ve |
| Optimum temp. (oC) | 32 | 37 | Sucrose | +ve | +ve |
| Indole | -ve | +ve | L-fructose | +ve | +ve |
| MR | -ve | +ve | L-rhamnose | +ve | +ve |
| VP | +ve | -ve | D-galactose | -ve | +ve |
| Starch hydrolysis | -ve | -ve | L-glucose | +ve | +ve  |
| Nitrate reduction | +ve | +ve | D-Trehalose | +ve | -ve |
| Amylase | +ve | -ve | L-mannose | -ve e | +ve |
| Hydrogen sulphide production | +ve | -ve | D-mannitol | +ve | -ve |

Table S2 Experimental design matrix of three levels of environmental factors with coded values by using Box-Behnken Design with observed responses

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Run | X1 | X2 | X3 | *Stenotrophomonas* sp. BAB-6435 | *Brevisbacillus brevis* BAB-6437 |
| DB (gL-1) | BP (gL-1) | Stability (E24%) | DB(gL-1) | BP(gL-1) | Stability (E24%) |
| 1 | 0 | 1 | 1 | 0.157 | 0.137 | 48.56 | 1.014 | 0.114 | 54.18 |
| 2 | -1 | 0 | -1 | 0.777 | 0.364 | 45.3 | 0.9147 | 0.541 | 63.41 |
| 3 | 0 | 0 | 0 | 1.37 | 0.936 | 80.78 | 3.217 | 2.115 | 88.88 |
| 4 | 0 | 0 | 0 | 1.97 | 0.918 | 81.25 | 3.364 | 2.084 | 89.27 |
| 5 | 0 | 0 | 0 | 1.788 | 0.924 | 81.08 | 3.088 | 2.154 | 89.79 |
| 6 | -1 | -1 | 0 | 0.677 | 0.372 | 80.76 | 2.1947 | 1.341 | 89.43 |
| 7 | 0 | 0 | 0 | 1.967 | 0.984 | 80.92 | 3.179 | 2.184 | 89.66 |
| 8 | -1 | 0 | 1 | 0.857 | 0.314 | 79.31 | 0.447 | 0.0431 | 88.24 |
| 9 | 0 | 1 | -1 | 0.177 | 0.0524 | 20.55 | 0.884 | 0.224 | 29.35 |
| 10 | 0 | 0 | 0 | 1.483 | 0.984 | 81.13 | 3.302 | 2.106 | 89.68 |
| 11 | 1 | 0 | -1 | 0.171 | 0.0184 | 48.96 | 0.589 | 0.0804 | 62.87 |
| 12 | 0 | -1 | -1 | 0.527 | 0.134 | 50.93 | 1.304 | 0.464 | 64.34 |
| 13 | 1 | -1 | 0 | 1.071 | 0.268 | 81.42 | 1.869 | 0.714 | 88.89 |
| 14 | -1 | 1 | 0 | 0.227 | 0.0604 | 47.38 | 0.77 | 0.101 | 54.44 |
| 15 | 0 | -1 | 1 | 0.607 | 0.484 | 81.94 | 1.434 | 0.114 | 65.17 |
| 16 | 1 | 1 | 0 | 0.621 | 0.2658 | 48.04 | 1.449 | 0.474 | 53.9 |
| 17 | 1 | 0 | 1 | 1.341 | 0.5684 | 79.97 | 1.719 | 0.804 | 87.7 |

X1-temperature; -1(4oC), 0(42oC), 1(80oC)

X2 –Salinity; -1(0mg/100ml), 0(5g/100ml), 1(10g/100ml

 X3-pH; -1(3.5), 0(8.5), 1(13.5)

DB-dry biomass of microbial cell; BP-biosurfactant production

Table S3 Level of significance for the response surface quadric model generated from Box behnken design (BBD)

|  |  |
| --- | --- |
| Source | ***P*-value**  |
| *Stenotrophomonas* sp. BAB-6435 | *Brevisbacillus brevis* BAB-6437 |
| DB | BP | Stability (E24%) | DB | BP | Stability (E24%) |
| Model | 0.003a | <0.0001a | < 0.0001a | < 0.0001a | <0.0001a | < 0.0001a |
| A | 0.279NS | 0.895NS | 0.0818NS | 0.0271c | 0.938NS | 0.8337NS |
| B | 0.021c | <0.0001a | < 0.0001a | 0.0007b | 0.0202c | < 0.0001a |
| C | 0.054NS | <0.0001a | < 0.0001a | 0.0885NS | 0.6959NS | < 0.0001a |
| AB | 1.00NS | 0.0017c | 1.0000NS | 0.0187c | 0.0435c | 0.8107NS |
| AC | 0.03c | <0.0001a | 0.1705NS | 0.0019c | 0.0198c | 0.4818NS |
| BC | 0.811NS | 0.0038c | 0.1705NS | 1.000NS | 0.5736NS | 0.0124c |
| A2 | 0.003c | <0.0001a | 0.0051c | <0.0001a | <0.0001a | 0.0231c |
| B2 | <0.0001a | <0.0001a | < 0.0001a | <0.0001a | <0.0001a | < 0.0001a |
| C2 | 0.0001a | <0.0001a | < 0.0001a | <0.0001a | <0.0001a | < 0.0001a |

a-significant at ≤0.0001; b- significant at ≤0.001; c-significant at ≤0.05; NS-non significant

DB-dry biomass; BP-biosurfactant production

A-temperature; B-NaCl; C-pH

Table S4 Micro-elemental analysis by Scanning electron microscopy-energy dispersive X-ray spectroscopy **(**SEM-EDS) of purified biosurfactants from *Stenotrophomonas sp.*BAB-6435 and *Brevisbacillus brevis* BAB-6437. Data are represented by weight and atomic % with their standard formula.

|  |  |  |  |
| --- | --- | --- | --- |
| **Element** | **Standard** | **Glycolipid** **(*Stenotrophomonas sp.* BAB-6435)** | **Lipopeptide** **(*Brevisbacillus brevis* BAB-6437)** |
|  |  | **Weight %** | **Atomic %** | **Weight %** | **Atomic %** |
| **C** | CaCO3 | 55.17 | 66.55 | 59.05 | 69.76 |
| **O** | SiO2 | 32.19 | 29.15 | 29.94 | 26.55 |
| **Na** | Albite | 1.78 | 1.12 | **-** | **-** |
| **P** | GaP | 1.88 | 0.88 | **-** | **-** |
| **S** | FeS2 | 3.19 | 1.44 | 5.75 | 2.55 |
| **K** | MAD-10 Feldspar | 1.42 | 0.53 | **-** | **-** |
| **Zn** | Zn | **-** | **-** | 5.26 | 1.14 |

**Figures**



Figure S1 Surface tension activity and CMC estimation of the Glycolipid () and Lipopeptide () produced by *Stenotrophomonas* sp.BAB-6435 and *Brevisbacillus brevis* BAB-6437 respectively. Cultures were grown in MSM media supplemented with 2.0% (w/v) glucose.

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Figure S2Foam formation profile of *Stenotrophomonas sp.* BAB-6435 (A) and *Brevisbacillus brevis* BAB-6437 (B) represented through foam height () and foaming index () at different time interval (0-24 hours) of culture incubation.



Figure S3 FT-IR spectrum of the purified biosurfactants; Glycolipid (A) and Lipopeptide (B) produced from *Stenotrophomonas* sp.BAB-6435 and *Brevisbacillus brevis* BAB-6437 respectively. Cultures were grown in MSM media supplemented with 2.0% (w/v) glucose.



Figure S4 Mass spectrum analysis of the GC profiling peaks of purified, derivatized Glycolipid biosurfactants. Spectrum identified as Palmitic acid methyl esters (a); Ribose (b); Galactose (c); and Maltose (d) using NIST mass spectrum database library.



Figure S5 Mass spectrum analysis of the GC profiling peaks of purified, derivatized Lipopeptide biosurfactants. Spectrum identified as Stearic acid methyl esters (a); Alanine (b); Glycine (c); Leucine (d); Proline (e); Serine (f); Aspartic acid (g) using NIST mass spectrum database library.