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9 **Predicting the minimum liquid surface tension activity of**
10 **pseudomonads expressing biosurfactants**

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22 **Keywords**

23 Biosurfactant, Liquid surface tension, *Pseudomonas*, Surfactant

25 **Running Headline**

26 Surface tension activity of biosurfactants

27 **SIGNIFICANCE AND IMPACT OF THE STUDY**

28 Numerous surveys of biosurfactant-producing bacteria have been conducted, but only
29 recently has an attempt been made to predict the minimum liquid surface tension these
30 surface-active agents can achieve. Here we determine a theoretical minimum of 24 mN
31 m^{-1} by statistical analysis of tensiometry data, suggesting a fundamental limit for
32 biosurfactant activity in bacterial cultures incubated under standard growth conditions.
33 This raises a challenge to our understanding of biosurfactant expression, secretion and
34 function, as well as being of interest to biotechnology where they are used in an
35 increasingly wide range of applications.

36

37 **ABSTRACT**

38 Bacteria produce a variety of biosurfactants capable of significantly reducing liquid
39 (aqueous) surface tension (γ) with a range of biological roles and biotechnological
40 uses. In order to determine the lowest achievable surface tension (γ_{Min}), we tested a
41 diverse collection of *Pseudomonas*-like isolates from contaminated soil and activated
42 sludge, and identified those expressing biosurfactants by drop-collapse assay. Liquid
43 surface tension reducing ability was quantitatively determined by tensiometry, with 57
44 isolates found to significantly lower culture supernatant surface tensions to 24.5 – 49.1
45 mN m^{-1} . Differences in biosurfactant behaviour determined by foaming, emulsion and
46 oil-displacement assays, was also observed amongst isolates producing surface
47 tensions of 25 – 27 mN m^{-1} , suggesting that a range of structurally-diverse
48 biosurfactants were being expressed. Individual distribution identification (IDI) analysis
49 was used to identify the theoretical probability distribution that best fitted the surface
50 tension data, which predicted a γ_{Min} of 24.24 mN m^{-1} . This was in agreement with
51 predictions based on earlier work of published mixed-bacterial *spp.* data, suggesting a
52 fundamental limit to the ability of bacterial biosurfactants to reduce surface tensions in

53 aqueous systems. This implies a biological restriction on the synthesis and export of
54 these agents or a physical-chemical restriction on their functioning once produced.

55

56 INTRODUCTION

57 Biosurfactants are surface active chemicals expressed by a range of organisms that
58 reduce liquid surface tensions (γ) of aqueous and aqueous-hydrocarbon (oil) mixtures.
59 Biosurfactants are widely used in biotechnology, including agriculture, cosmetics, food,
60 pharmacology, bioremediation and oil recovery (see reviews by Ron and Rosenberg,
61 2002; Mulligan, 2005; Marchant *et al.*, 2012; Ławniczak *et al.*, 2013; Sachdev and
62 Cameotra, 2013), whilst the biological roles for bacterial biosurfactants include motility
63 and virulence, the inhibition of nematode and protist predation, lysis of fungi and
64 oomycetes, and the induction of systemic resistance in plants (reviewed by
65 Raaijmakers *et al.*, 2010), as well as modifying water distribution in soil pore networks
66 (Fechtner *et al.*, 2011).

67 Bacterial biosurfactant expression is readily surveyed using modifications of the
68 drop-collapse assay (Persson and Molin, 1987), blood agar, oil plates and oil spays,
69 and quantified by tensiometry of cultures or purified preparations (e.g. Bodour *et al.*,
70 2003; Youssef *et al.*, 2004; Burch *et al.*, 2010). Surfactants can be behaviourally
71 characterised by foaming, emulsion and oil-displacement assays, and are known to be
72 structurally diverse, including glycolipids, lipopeptides, lipopolysaccharides, etc.
73 (Marchant *et al.*, 2012; Ławniczak *et al.*, 2013; Sachdev and Cameotra, 2013).

74 The process from the isolation of biosurfactant expressing bacteria through to
75 the chemical-structural analysis of biosurfactants is time-consuming, and a recent
76 evaluation of the liquid surface tension reducing ability (LSTRA) of environmental
77 pseudomonads by Individual distribution identification analysis suggests that
78 biosurfactant activity may be limited (γ_{Min}) to 24 mN m⁻¹ (Fechtner *et al.*, 2011). The first
79 bacterial biosurfactant to be characterised, surfactin, expressed by *Bacillus subtilis*,

80 could reduce γ to 27 mN m⁻¹ (Peypoux *et al.* 1999). Perhaps surprisingly, since then
81 significantly higher activities producing lower surface tensions have not been reported,
82 despite the isolation and characterisation of many more biosurfactants from a range of
83 different bacteria (e.g. 22 – 25 mN m⁻¹ reported by Morikawa *et al.*, 1993; Nielsen *et al.*,
84 2002; Kuiper *et al.*, 2004; Fechtner *et al.*, 2011; Xie *et al.*, 2011; Saimmai *et al.*, 2012).
85 A limit to biosurfactant activity suggests a biological restriction in the synthesis of these
86 agents or a need to prevent self-damage during expression (Fechtner *et al.*, 2011). *In*
87 *extremis*, surfactant absorption to the air-liquid interface may be kinetically limited, and
88 under these conditions the physical-chemical properties of the solution and
89 atmosphere will also be important.

90 In this report, we sought to confirm γ_{Min} by examining an independent set of
91 pseudomonads recovered from soil and activated sludge, and a parallel set of recently
92 published bacterial LSTRA measurements. Furthermore, we uncover substantial
93 variation in biosurfactant behaviour within groups of isolates with very similar LSTRA,
94 suggesting that these isolates are expressing a range of structurally-diverse
95 biosurfactants.

96 97 **RESULTS AND DISCUSSION**

98 We obtained a diverse bacterial collection of 355 *Pseudomonas* or *Pseudomonas*-like
99 isolates from activated sludge and soil samples by selection for aerobic growth on
100 PSA-CFC plates. A preliminary analysis of isolate phenotype data by cluster analysis
101 (after Robertson *et al.*, 2013) demonstrated that the collection was diverse with few
102 biological replicates. The collection was screened for LSTRA by drop-collapse assay of
103 18 h early stationary-phase KB cultures (after Persson and Molin, 1987), identifying 71
104 isolates (20% of the total) likely to be expressing biosurfactants under the conditions
105 used here.

106 The positive isolates, plus 14 randomly-chosen drop-collapse–negative isolates,
107 were investigated further by quantitative tensiometry of cell-free 24 h stationary-phase
108 shaken KB culture supernatants (Figure 1). Significant differences were found between
109 isolates (ANOVA, $P < 0.001$), with 57 LSTRA isolates found to significantly reduce the
110 liquid surface tension of sterile KB from 53 mN m^{-1} and 28 that did not (the non-LSTRA
111 group) (Dunnett's method, $\alpha = 0.05$).

112 We note that 18 drop-collapse–positive isolates did not show significant LSTRA
113 in culture supernatants whilst 4 drop-collapse–negative isolates did, indicating that the
114 drop-collapse assay is not always reliable and that biosurfactant expression may be
115 sensitive to growth conditions, as has been reported earlier (Burch *et al.*, 2011;
116 Fechtner *et al.*, 2011). However, increasing incubation periods from 24 h to 48 h and
117 maintaining cultures in the stationary phase for longer made little difference to final
118 surface tensions, with only two of eight strains tested across the non-LSTRA,
119 intermediate- γ and low- γ LSTRA groups showing significant but minor decreases in
120 surface tensions of $2 - 5.3 \text{ mN m}^{-1}$ (*t*-tests, $P \leq 0.05$) (see *Supplementary Table S1*);
121 more often surface tension increased by $0.3 - 4.1 \text{ mN m}^{-1}$ ($P \leq 0.05$), presumably due
122 to the effects of culture aging and cell lysis (Fechtner *et al.*, 2011).

123 In order to predict the minimum bacterial biosurfactant activity (γ_{Min}) in cultures,
124 we analysed the surface tension data for 50 LSTRA isolates that formed a single large
125 homogeneous group (Isolates #69 - #67 as shown left to right in Figure 1), excluding
126 the remaining 7 LSTRA isolates as they showed a poorer ability to reduce surface
127 tension and appeared statistically to be an extension of the non-LSTRA group (TK-
128 HSD, $\alpha = 0.05$). Individual distribution identification (IDI) analysis found that gamma,
129 log-normal and log-logistic distributions fitted the surface tension data well, with the
130 best-fit provided by a log-logistic distribution based on the Anderson-Darling goodness
131 of fit test (AD = 0.497, $P = 0.293$) predicting γ_{Min} of 24.24 mN m^{-1} (Table 1). This
132 compares well with predictions made from a collection of soil pseudomonads (Fechtner

133 *et al.*, 2011) and from a random sample of published reports of LSTRA from a range of
134 bacteria including non-pseudomonads (Table 1; see also *Supplementary Table S2* and
135 references therein). We are not aware of any bacterium able to reduce the liquid
136 surface tension of cultures significantly below 22 – 25 mN m⁻¹ (Morikawa *et al.*, 1993;
137 Nielsen *et al.*, 2002; Kuiper *et al.*, 2004; Fechtner *et al.*, 2011; Xia *et al.*, 2011;
138 Saimmai *et al.*, 2012) and the theoretical 24 mN m⁻¹ limit identified by Fechtner *et al.*
139 (2011) and confirmed by the work reported here (we note that the lowest value of 22.56
140 mN m⁻¹ given by Xia *et al.* (2011) is reported as a single measurement with no
141 indication of reproducibility). Although biosurfactant activity is concentration,
142 temperature and solution dependant, we do not believe that the theoretical limit
143 determined here is unique to KB culture supernatants, as other reports of biosurfactant
144 surface tensions in the 22 – 25 mN m⁻¹ range have been for a variety of spent culture
145 media and solutions of purified surfactant. It is important to note that in bacterial
146 cultures the critical micelle concentration (CMC), corresponding to the lowest surface
147 tension produced by a biosurfactant, may not be achieved for a number of reasons,
148 including growth limitations and biosurfactant interactions with other compounds.
149 Further research is necessary to determine whether the theoretical limit is the same for
150 purified biosurfactant solutions; if it is, then we would suggest that there is a biological
151 restriction on the expression of more active biosurfactants, but if not, we would suggest
152 that the limit is at least partially restricted by the biochemical system in which
153 biosurfactants and other compounds interact.

154 We were interested in determining whether there is evidence of structural
155 diversity amongst the biosurfactants produced by the low- γ LSTRA isolates, as this
156 could be used to select isolates for further chemical-structural analyses and testing of
157 novel biosurfactants. We approached this by identifying two homogeneous groups of
158 isolates producing significant, but small differences in surface tensions □□ Group I:
159 25.2 □□□ 0.1 mN m⁻¹ □□□□□ Group II: 26.7 □□□ 0.5 mN m⁻¹; TK-HSD, $\alpha = 0.05$). We

160 then investigated the biosurfactant behaviours of these isolates using emulsion,
161 foaming and oil-displacement assays (see Table 2 listing the behaviours of these and
162 other low- γ LSTRA isolates). Cluster analysis of the behaviour data showed significant
163 variation between isolates, suggesting that there may be structural diversity in the
164 biosurfactants they express (Figure 2). This analysis failed to cluster the isolates into
165 the original set of two groups, indicating that both groups were likely to be producing a
166 similar set of diverse biosurfactants. The difference between Group I and II surface
167 tensions cannot be explained by distinct types of biosurfactant, as surface tension and
168 behaviour were not significantly associated (Fisher's exact test, $P = 0.1201$). In
169 contrast, surface tension and isolate phenotype (determined using a number of
170 enzymatic and growth-based assays) were weakly associated ($P = 0.0768$), suggesting
171 that closely-related isolates may express more similar biosurfactant types than
172 distantly-related isolates.

173

174 **CONCLUDING STATEMENT**

175 Surveying environmental bacterial isolates for the expression of biosurfactants is
176 relatively straight forward using a series of techniques such as the drop collapse assay,
177 blood agar, oil plates and oil sprays, followed by the quantification of surface tension by
178 tensiometry. However, there appears to be a fundamental limit to the reduction of liquid
179 surface tension of bacterial cultures of 24 mN m^{-1} , suggesting that the hunt for more
180 active agents will become progressively less rewarding. Nonetheless, within very
181 narrow ranges of liquid surface tension, there is evidence for bacterial biosurfactants
182 with substantially different behaviours that may be of greater interest in biotechnology
183 applications than the absolute surface tension that can be achieved.

184

185

186 **MATERIALS AND METHODS**

187 Bacteria were isolated from activated sludge samples from the Hatton Wastewater
188 Treatment Plant at Arbroath and soil samples from a roadside site near Dundee with a
189 history of hydrocarbon contamination by growth on *Pseudomonas*-selective agar plates
190 (PSA-CFC, Oxoid, UK) under aerobic conditions for 72 h at 20°C. Randomly-chosen
191 colonies were re-streaked on PAS-CFC plates before being used to inoculate over-
192 night KB shaken cultures (King's B; 10 g glycerol, 1.5 g K₂HP0₄, 1.5 g MgSO₄.7H₂O and
193 20 g Proteose peptone No. 3 (Becton, Dickinson and Company, UK) per litre) and
194 aliquots of these maintained at -80°C as 15% (v/v) glycerol stocks. Isolates were
195 phenotypically characterized using a number of enzymatic and growth-based assays
196 after Robertson *et al.* (2013) (see *Supplementary Information* for further details), and a
197 drop collapse assay (Persson and Molin, 1987) used to screen isolates for those
198 expressing biosurfactants in 18 h early-stationary-phase shaken KB cultures.

199 The liquid surface tension (γ) of replicate cell-free shaken 24 h stationary-phase
200 shaken KB culture supernatants ($n = 4$) were quantitatively measured using a K100 Mk
201 2 Tensiometer (Krüss, Germany) by the rod method at 20°C as described by Fechtner
202 *et al.* (2011) (using this method, γ_{water} was $73.2 \pm 0.1 \text{ mN m}^{-1}$). Data were examined by
203 *t*-tests, ANOVA, and *post hoc* multiple comparison tests including Dunnett's method
204 with a control and Tukey-Kramer HSD (TK-HSD) (JMP 7, SAS Institute, USA).
205 Individual distribution identification (IDI) analysis based on the Anderson-Darling (AD)
206 goodness of fit test was used to identify theoretical probability distributions to fit surface
207 tension data and predict γ_{Min} using the threshold parameters of the fitted distributions
208 (MINITAB v.15, Minitab Ltd, UK). Published surface tension data for 59 mixed bacterial
209 *spp.* was also analysed by IDI (see *Supplementary Information* for further details).

210 Biosurfactant behaviour was assessed by foaming assay (after Sathe and
211 Salunke, 1981), and emulsion and oil-displacement assays (Youssef *et al.*, 2004;
212 Prieto *et al.*, 2008) (see *Supplementary Information* for further details). Biosurfactant
213 behaviours were investigated by pairwise correlations (r^2) and cluster analysis using a

214 hierarchical approach and Ward's minimum variance method (JMP 7). The association
215 between surface tension, phenotype and surfactant behaviour data was assessed
216 using a 2 x 2 contingency table approach and Fisher's exact test (see *Supplementary*
217 *Information* for further details).

218

219

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226

227 **CONFLICT OF INTEREST**

228 No conflict of interest declared.

229

230 **SUPPORTING INFORMATION**

- 231 • Materials and methods for the emulsion, foaming and oil-displacement assays,
232 including the selection of published reports of bacterial surface tension
233 measurements and the association analysis.
- 234 • List of phenotype assays used in this work.
- 235 • Table S1: Differences in liquid surface tension at 24 h and 48 h.
- 236 • Table S2: Bacterial liquid surface tension reducing activity (LSTRA).
- 237 • References for Supplementary Table S2.

238

239

240

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322

323 **FIGURE LEGENDS**

324 **Figure 1. Contaminated soil and activated sludge-isolated pseudomonads**
325 **express liquid surface tension-reducing activity.** A total of 57 of 85
326 isolates significantly reduced the liquid surface tension (γ_{\square}) of sterile King's
327 B (KB) medium from $52.8 \pm 0.5 \text{ mN.m}^{-1}$ (dashed line) to $24.5 \pm 0.1 - 49.1 \pm$
328 1.4 mN m^{-1} , whilst the remaining 28 isolates did not (Dunnett's method
329 using γ_{\square} as the control, $\alpha = 0.05$) (left to right, LSTRA isolates #69 – #75
330 and non-LSTRA isolates #114 – #311, respectively). The LSTRA isolates
331 are further differentiated into the low and intermediate- γ groups as
332 indicated. Liquid surface tension was determined by tensiometry of cell-free
333 supernatants produced from 24 h stationary-phase shaken KB culture
334 cultures. Mean and standard errors ($n = 4$) are shown. The bimodal
335 distribution of surface tension measurements is shown in the inset
336 histogram.

337

338 **Figure 2. The low- γ LSTRA isolates show evidence of variation in biosurfactant**
339 **behaviour.** Cluster analysis of the biosurfactant behaviour data for the two
340 homogeneous groups of isolates producing different surface tensions
341 (Group I, circles; and II, squares) demonstrates significant variation
342 between isolates and an overlap (mixing) of the two groups. Biosurfactant
343 behaviours were determined by foaming, emulsion and oil-displacement
344 assays (see Table 2), and isolates are linked on the basis of similarity. The
345 dendrogram is shown with an even-spacing horizontal scale.

346

347 **TABLES**

348 Table 1. A comparison of predicted γ_{Min} determined by IDI analysis.

349		Best-fitting	P□□□□□□□□			
350		3-parameter				
351	γ_{Min}					
352	Data set	distribution*	N	P	AD	(mN m ⁻¹)
353	<hr/>					
354	Contaminated soil and activated sludge–isolated pseudomonads	Log-logistic	50	0.294	0.497	24.24
355						
356	Soil–isolated pseudomonads (Fechtner <i>et al.</i> , 2011)	Gamma	38	0.233	0.688	24.16
357						
358	Random sampling of published reports for mixed bacterial spp.†	Weibull	59	0.386	0.238	24.23
359	<hr/>					

360 Individual distribution identification (IDI) analyses were used to fit theoretical probability distributions to
 361 surface tension data, and from the threshold parameters to predict γ_{Min} . N, Number of bacterial isolates; P,
 362 P-value; AD, Anderson-Darling test statistic; *, For all data sets, gamma, log-normal and log-logistic
 363 distributions fitted the surface tension data well; additionally, the Weibull distribution and the Johnson
 364 transformation of a normal distribution fitted the Random sampling of published reports data well; †, A
 365 description of the selection of set of bacteria and a list of bacteria, surface tensions and references are
 366 provided in the Supplementary Information (only those with surface tensions ≤ 42.1 mN m⁻¹ were used in
 367 this analysis, following the upper limit of LSTRA determined here for the contaminated soil and activated
 368 sludge–isolated pseudomonads).

369
 370
 371

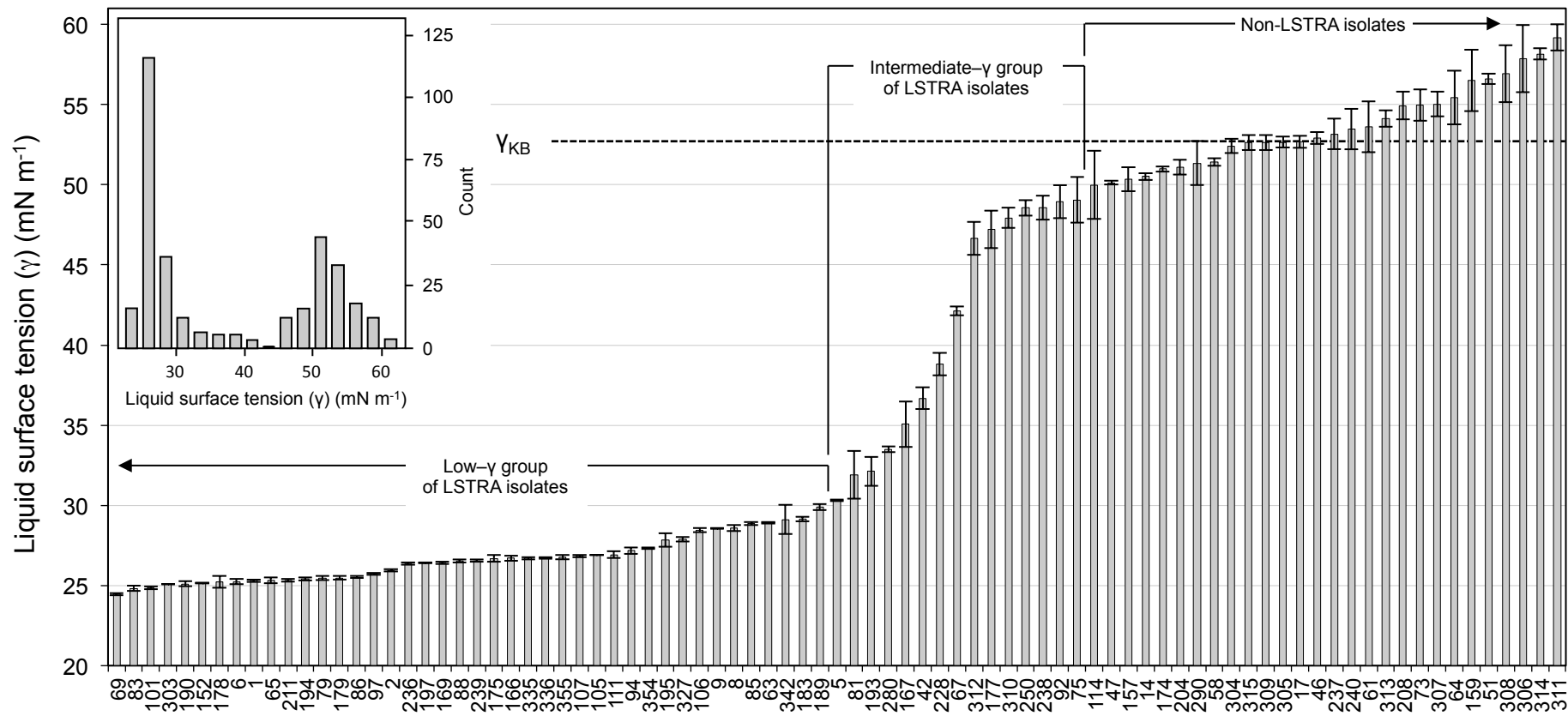
Manuscript in publication

372 Table 2. Biosurfactant behaviours of low- γ LSTRA isolates.

373		Liquid surface tension ^a (mN m ⁻¹)	Emulsion Assay ^b			Foam stability ^c (%)	Oil- displacement ^d (mm)
374	Isolate		O _i	A _i	E _i		
375	69	24.5 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	30.0 ± 0.0
376	83	24.8 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	39.7 ± 2.0
	101	24.9 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.1 ± 0.1	10.0 ± 0.6
	303	25.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	30.0 ± 0.0
	190	25.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	24.3 ± 0.7
	152	25.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.2 ± 0.1	30.0 ± 2.9
	178	25.2 ± 0.4	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	27.7 ± 1.2
	6	25.2 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	31.0 ± 0.6
	1	25.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	34.0 ± 1.5
	65	25.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.7 ± 0.0	0.3 ± 0.1	46.3 ± 1.9
	211	25.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	41.3 ± 1.9
	194	25.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	15.0 ± 0.6
	79	25.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	8.3 ± 0.9
	179	25.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	8.3 ± 0.9
	86	25.5 ± 0.1	0.5 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	44.0 ± 1.5
	97	25.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.6 ± 0.0	0.2 ± 0.1	29.0 ± 0.6
	2	25.9 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.3 ± 0.1	13.0 ± 0.6
	236	26.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	42.7 ± 2.3
	197	26.4 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	10.0 ± 0.6
	169	26.4 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.1	30.7 ± 1.8
	88	26.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	8.0 ± 0.6
	239	26.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.1	29.7 ± 0.3
	175	26.7 ± 0.2	0.2 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.1	2.3 ± 0.3
	166	26.7 ± 0.2	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.3 ± 0.1	21.0 ± 0.6
	335	26.7 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.1 ± 0.1	3.3 ± 0.3
	336	26.7 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.1	43.7 ± 0.9
	355	26.8 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	37.3 ± 1.5
	105	26.8 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	15.3 ± 0.3
	107	26.9 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	1.0 ± 0.6
	111	26.9 ± 0.2	0.0 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.4 ± 0.1	8.0 ± 0.6
	94	27.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.7 ± 0.0	0.2 ± 0.0	8.0 ± 0.6
	354	27.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.1	34.3 ± 2.8
	195	27.8 ± 0.4	0.1 ± 0.0	0.2 ± 0.1	0.7 ± 0.0	0.2 ± 0.1	2.3 ± 0.3
	327	27.9 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	1.0 ± 0.0
	106	28.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	11.0 ± 0.6
	9	28.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	20.7 ± 1.2
	8	28.6 ± 0.2	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	14.0 ± 0.6
	85	28.9 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	10.7 ± 0.7
	63	28.9 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	34.0 ± 0.6
	342	29.1 ± 0.9	0.0 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	2.0 ± 0.0
	183	29.2 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.7 ± 0.1	0.2 ± 0.0	43.0 ± 0.6

418 The surfactant behaviours of the first 41 low- γ LSTRA isolates were determined and listed here in increasing surface tension order (as shown in Fig. 1). Means
419 and standard errors are shown. a, Liquid surface tensions were determined by tensiometry of cell-free 18 h KB cultures ($n = 4$); b, Oil (O_i), aqueous (A_i) and
420 emulsion (E_i) indices were determined as the relative height of each layer after shaking a mixture of 18 h KB culture and oil after 24 h ($n = 3$); c, Foam stability
421 was determined as the percentage reduction of foam heights after 2 h using 18 h KB cultures ($n = 3$); d, The displacement of an oil film by a drop of 18 h KB
422 culture was measured as the drop diameter (mm) after 5 s ($n = 3$). Significant correlations were observed between A_i x E_i ($r^2 = -0.4510$, $P = 0.0031$), O_i x E_i ($r^2 =$
423 -0.7295 , $P < 0.0001$), Oil-displacement x E_i ($r^2 = -0.3368$, $P = 0.0313$) and Oil-displacement x O_i ($r^2 = 0.3867$, $P = 0.0125$); all other pair-wise correlations were
424 not significant ($P \leq 0.05$).
425

426



Contaminated soil and activated sludge-isolated pseudomonads

Figure 1

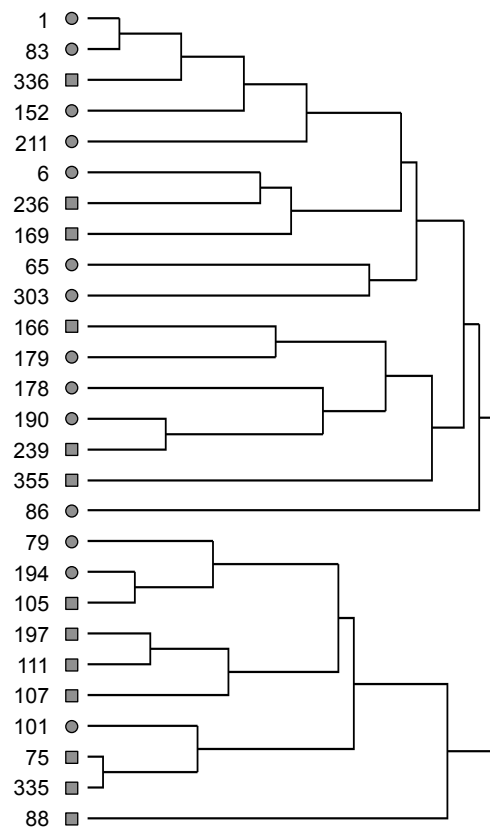


Figure 2