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LIMITATION OF BIOSURFACTANT STRENGTH PRODUCED BY BACTERIA

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Abstract

Biosurfactants which reduce the surface or interfacial tension of liquids and act as emulsifiers, foaming and dispersing agents with low toxicity, are finding increasing applications in biotechnology and driving the search for novel compounds for further exploitation. Potential biosurfactants sourced from bacteria are often selected first by qualitative assessment of surface tension using simple assays such as the drop collapse technique or by quantitative tensiometry measuring air-liquid interfacial tension of cell-free culture supernatants or purified samples, and subsequently tested for appropriate physical-chemical behaviours using a range of application-specific assays. Highly active or strong biosurfactants have been reported to reduce the surface tension of water to approximately 22 – 25 $mN.m^{-1}$, and show a range of behaviours determined by the choice of conditions (temperature, pH, salt concentration, etc.) used to test particular aqueous-hydrophobic (oil) mixtures. However, recent analyses of biosurfactant strengths using a predictive statistical approach (Individual distribution identification) have shown that it is unlikely that new compounds will be identified able to significantly reduce aqueous surface tensions below 24 $mN.m^{-1}$. The mechanistic basis of this limit requires an explanation of why stronger compounds are not produced by bacteria, with a limitation of self-harm to producing cells probably the most likely biophysical explanation. However, behavioural analyses using a combination of emulsion, foam stability and oil-dispersion assays indicates high chemical diversity exists amongst biosurfactants exhibiting the strongest levels of activity (24 – 28 $mN.m^{-1}$), suggesting that bacteria are still likely to provide a rich source of potentially novel compounds for use in biotechnology.

Introduction

Biosurfactants produced by bacteria have a range of roles in different environments as well as increasingly-important uses in biotechnology (for a selection of reviews, see [7, 21, 32, 39, 40, 50, 53, 55, 56]). Microbial biosurfactants can be structurally classified into four major classes (glycolipids, lipopeptides/lipoamino acids, polymers including proteins and polysaccharides, and oil/membranes including lipids and fatty acids) (cited in [39]). Both biosurfactant-producing bacteria and the surface-active compounds they express are most usually identified through surveys of bacterial collections or isolates from particular environments, using simple qualitative assays such as the drop collapse technique where the shape of a drop of culture on a glass or plastic surface is assessed. Strains found to be positive for biosurfactant-production are then characterised further, often to the extent of semi-purifying and testing culture supernatants, and sometimes as far as determining the chemical structure of purified compounds. In parallel, the surface activity of biosurfactants is often determined quantitatively by tensiometry measuring the air-liquid interfacial tension (more commonly referred to as the liquid surface tension), and cultures or semi-purified material then tested using assays specific for particular biotechnological applications of interest. Here we also refer to compounds showing high surface activity as ‘strong’ biosurfactants, and bacteria producing these show significant liquid surface tension reducing abilities [16].

However, the process of identifying novel biosurfactants for biotechnology necessarily starts with a large collection of bacterial strains which are screened with simple qualitative assays to identify biosurfactant-producers, and then by increasingly more specific, time-consuming and expensive quantitative assays, to identify a small number of candidates for pilot-scale testing in which the commercial value of novel biosurfactants can be assessed.

The first biosurfactant to be isolated from bacteria was a cyclic lipopeptide referred to as surfactin, expressed by *Bacillus subtilis* and capable of reducing the liquid surface tension of water from 72 $mN.m^{-1}$ to 27 $mN.m^{-1}$ [6, 23, 24] (reviewed by [46]). Since then, a range of biosurfactants produced by bacteria, mainly by *Bacillus* and *Pseudomonas* spp., have been reported to reduce liquid surface tensions even more down to 22 – 25 $mN.m^{-1}$ (e.g. [11, 16, 17, 22, 26, 27, 28, 35, 36, 38, 42, 48, 57, 60, 67]). However, it is not clear whether substantially stronger biosurfactants are being found in new surveys, as it is hard to compare work carried out over the past 30 – 40 years due to the difficulty of accessing such a large body of literature (see [39] for an illustration of the increase in biosurfactant papers in this period). Furthermore, biosurfactant activities are more generally compared within studies, whilst biology or biotechnology–focussed reviews are not generally representative and are rapidly out-dated. For example, a limit of approximately 29 $mN.m^{-1}$ was suggested in a 2012 review [32] based on an earlier 1997 article listing forty-six research papers [14].

We have addressed this question by taking a statistical approach based on Individual Distribution Identification (IDI) to determine whether a limit to biosurfactant strengths exists, based on our own surveys of collections of bacteria and of published reports, and if so, how close have surveys got to this limit (it is important to note that the limit discussed here is for all bacterial biosurfactants, and is not the same as the limit a particular biosurfactant approaches as the concentration closes on the critical micelle concentration [21]). We are also interested to determine the extent of behavioural diversity amongst the strongest group of biosurfactants in order to determine whether one chemical-structural class of biosurfactants is stronger than all other classes, and because a variety of strong biosurfactants with a range of behaviours are clearly required for future biotechnological applications. To illustrate this point, we consider the biosurfactant strengths that might be found in a hypothetical collection of phylogenetically diverse bacteria expressing a range of different biosurfactants (**Figure 1**). Clearly, there must be a limit to the liquid surface tension of culture media that could be used to grow bacteria and produce biosurfactants (the liquid surface tension of aqueous solutions can be significantly reduced by the addition of solvents and solutes, but often at concentrations that would prevent bacterial growth; we have used media with liquid surface tensions of 41 $mN.m^{-1}$ (Lauria broth), 47 – 53 $mN.m^{-1}$ (King's B) and 60 $mN.m^{-1}$ (Minimal M9 Glucose) [16, 35]). Below the liquid surface tension of the culture media we envision a series of downward 'steps' corresponding to the expression of a particular biosurfactant or a group of structurally closely-related homologues (i.e. a class or sub-class of biosurfactants). In this hypothetical case, the width of individual step treads would reflect the number of strains producing a particular biosurfactant, class of biosurfactants, or a mix of different biosurfactants; the first might result from a group of phylogenetically closely-related strains, the second from more distantly-related strains in which sequence diversification has resulted in minor modifications of the ancestral biosurfactant, and the third resulting from strains expressing un-related biosurfactants having the same surface activities. This stepped-pattern

also recognises the fact that many compounds expressed by bacteria may have weak surface activities yet are insufficiently strong to be considered a biosurfactant (there is no generally agreed level for surface activity below which a compound is recognised as a biosurfactant, but 30 – 40 $mN.m^{-1}$ seems to be a reasonable threshold, e.g. [40]). We also note that in aging bacterial cultures liquid surface tensions can be significantly increased, suggesting that some bacteria produce 'anti-surfactants' [16, 35].

– FIGURE 1 TO GO NEAR HERE –

However, in several surveys of bacterial collections [16, 35] and in on-going work (**Figure 2**), we have found very smooth distributions of biosurfactant strengths. This may be a result of within–strain (replicate) variation in measurements, interactions between biosurfactants and other compounds differentially expressed by strains, as well as a more relevant and interesting diversity within and between biosurfactant classes.

– FIGURE 2 TO GO NEAR HERE –

Estimating a limit to biosurfactant strength

Biosurfactant strengths can also be examined using a statistical approach which aims to identify a probability distribution model that provides the best fit to the observed data [10]. Different statistical packages such as Minitab (Minitab Inc.), Matlab (The Mathworks Inc.), or SAS (SAS Institute Inc.) have implemented tools for probability distribution fitting tools. In Minitab (v.16.) this is done via the Individual Distribution Identification (IDI) tool which has the capability to fit sixteen individual distributions including the normal, log-normal, exponential, two or three parameter gamma, and two or three parameter Weibull distribution as well as Johnstone transformation for normal distribution fit [37]. As part of this tool the Anderson-Darling (AD) goodness of fit test is used for best distribution model selection based on the test statistic and the corresponding p-value (a god fit is reflected in a low AD test statistic and a high p-value). A lower limit to the expected biosurfactant strength can be then calculated from the model parameter estimates.

We have used this approach to estimate the the lower limit of biosurfactant strengths from a number of surveys of bacteria in which we determined liquid surface tensions from cell-free culture supernatants or obtained similar data from research publications. We originally screened a comprehensive collection of environmental pseudomonads we had acquired during other research, including many plant pathogens, plant and soil-associated strains, as well as a set of pseudomonads directly isolated from sandy loam soil [16], and have continued with this type of analysis with a more diverse collection of pseudomonads or *Pseudomonas*–like bacteria isolated from activated sludge from a waste-water treatment system and oil-contaminated soil [35]. Our current research is focussed on a small number of fluorescent pseudomonads recovered from directly below the roots of grass growing in uncontaminated soil. IDI analyses of these data all predict a similar lower limit for bacterial biosurfactant strength (γ_{Min}) of 24 $mN.m^{-1}$ (**Table 1**). This limit is probably not specific to pseudomonads,

as an analysis of surface tension strengths published for fifty-nine bacterial strains including eight *Bacillus* spp. results in a similar prediction [35]. Our review of the literature suggests that surface tensions for high-strength bacterial biosurfactants range from 22 – 25 $mN.m^{-1}$ (see [35] and references therein), with the lowest resulting from an un-replicated measurement with no indication of reproducibility [67]. We note that recent publications have not listed any stronger biosurfactants (**Table 2**), and as a result, we remain confident of our prediction of the lower limit for bacterial biosurfactant strength.

– TABLE 1 TO GO NEAR HERE –

– TABLE 2 TO GO NEAR HERE –

Mechanistic basis of the limit

The mechanism or mechanisms limiting the production of stronger biosurfactants currently lacks a detailed explanation, although we suggest that a limitation of self-harm to producing cells is probably the most likely biophysical explanation. All biosurfactants are synthesised in the cytosol though ribosomal or non-ribosomal peptide synthesis [18] and specific biosynthetic enzyme activity, and at each stage, intermediates or the final compounds could have a detrimental biophysical effect on the producing cell.

Lipopeptide biosurfactants, such as fengicin, iturin and surfactin, produced by *Bacillus* spp. and amphicin, syringomycin and viscosin, produced by *Pseudomonas* spp. [54] are synthesised via relatively unusual non-ribosomal peptide synthase (NRPS) enzyme complexes [34]. These enzyme complexes are encoded by gene clusters and are modularly organised, allowing different modules to combine in the production of different biosurfactants. These complexes drive the amino acid addition of the peptide portion of lipopeptide biosurfactants to a fatty acid moiety produced from cellular fatty acid metabolism, in an amino to carboxy-terminus direction with cyclisation in some lipopeptides [34, 54]. Global regulation of these complexes is controlled at a transcriptional level by two-component systems such as ComP/ComA and DegS/DegU in *Bacillus* spp. [33, 66] and similar two-component systems in *Pseudomonas* spp. [25, 41] to control production of these biosurfactants depending on the stage of the cell cycle and environmental conditions.

Glycolipid biosurfactants, such as the rhamnolipids produced by *Pseudomonas* spp. and trehalose lipid by *Rhodococcus* spp., are synthesised by specific enzymes. Lipid components of glycolipids proceed through the classical fatty acid synthesis, where, in the example of rhamnolipids, the β-hydroxydecanoyl-ACP intermediate from the FASII cycle is sequestered by RhlA, and RhlB and RhlC then catalyse the transfer of rhamnose from dTDP-rhamnose to form mono or di-rhmanolipids [3, 51]. RhlAB is both transcriptionally and post-transcriptionally regulated by factors related to quorum sensing and is also involved in swarming and biofilm formation [12, 31, 51]. The dTDP-rhamnose itself is synthesised in bacteria from glucose by RmlABCD and is transcriptionally auto-regulated by RmlA [51].

The chemical nature of biosurfactants makes them inherently amphiphilic with fatty acid moieties which are hydrophobic, have an affinity for other amphipathic molecules (i.e. the major components of biological membranes) through hydrophobic or other interactions, and have demonstrated toxicity to bacterial cells [30, 65]. The physicochemical nature of biosurfactants is the most likely mechanism limiting the production of stronger compounds, creating a selective pressure by which the development of stronger biosurfactants leads to unsustainable self-damage, and is therefore genetically and phenotypically unfavourable.

The major phospholipid and membrane component in bacteria is phosphatidylethanolamine, with some bacterial membranes also containing phosphatidylcholine [61]. Bacteria which produce biosurfactants must therefore have membranes containing these molecules which are not disrupted by the levels of biosurfactants they produce inside and around the cell. Lipopeptide biosurfactants have been shown to lead to the permeabilisation of biological membranes [9], by interacting with phospholipid acyl chains and causing the formation of ion-conducting pores [8, 19]. This disrupts membrane integrity causing leakage and effecting normal cell function [8, 13, 19, 20]. Indeed, many lipopeptides are categorised as antibiotics, with some molecules used as clinical antimicrobials (e.g. daptomycin and polymyxins) [47], because they kill bacterial cells via membrane disruption. Alteration of the cell surface including changes to lipopolysaccharide (LPS) and lipid A leads to reduced sensitivity to polymyxin antimicrobials [43], suggesting that biosurfactants directly damage biological membranes, with stronger molecules potentially disrupting membrane function further.

Glyocolipids, particularly the rhamnolipids, have been more widely studied to understand the effects they have on cells and cell membranes. Studies in a variety of different bacteria, including *Pseudomonas* and *Bacillus* spp., and model membranes, have demonstrated that the rhamnolipids alter the biophysical properties of membranes leading to destabilisation and permeabilisation [1, 2, 58, 59, 62, 63]. More detailed studies on the effects of rhamnolipids on *Pseudomonas aeruginosa* have demonstrated a marked decrease in the levels of major outer membrane proteins OprDFJM, leading to a thinner and more compact structure [64] that resists the permeabilisation and destabilisation of the membrane. A reduction in these outer membrane proteins would lead to less regulation molecules and ions moving across the membrane and reduce structural integrity. Exposure to rhamnolipid also leads to a decrease in LPS by solubilisation and by complexing Mg^{2+} [4, 64]. LPS is a major component of Gram-negative bacteria outer membranes, contributing to the structural integrity of the cell and stabilising the membrane. Trehalose lipid has also been shown to disrupt membranes through interactions with phosphatidylethanolamine and phosphatidycholine, altering lipid fluidity which is an essential component of bacterial membrane function [5, 44].

Together these observations suggests that stronger biosurfactants may have an increased ability to disrupt membranes and cause self-harm in the bacteria producing them, leading to a strong evolutionary selection disadvantage which has limited their emergence. However, there are other mechanisms which may limit the production of stronger biosurfactants. One potential mechanism is the increased ability of stronger biosurfactants to solubilise or disrupt proteins or other macromolecules inside cells which could interrupt essential metabolic pathways or secretion systems; these may be indirectly or directly linked to biosurfactant production, disruption of secretion mechanisms might also lead to the accumulation of

intermediates or products which may be toxic. The production of stronger biosurfactants might also be restricted by community interactions of producer cells in biofilms, with stronger compounds disrupting cell signalling or reducing surface tension to a point where biofilms are weak or poorly formed.

Evidence for chemical diversity amongst the strongest biosurfactants

We are also interested in determining the extent of chemical diversity amongst the strongest group of bacterial biosurfactants and are focussing on a group of fluorescent pseudomonads which produce liquid surface tensions in the range of 24 – 26 $mN.m^{-1}$ in cell-free culture supernatants. Although this group may be phylogenetically similar, the pseudomonads are known to produce several different classes of biosurfactants, including the cyclic lipo-peptides and rhamnolipids [39, 50]. Ultimately the best way to assess biosurfactant diversity would be to purify compounds and determine chemical structures, but this approach even for a single example is both expensive and time-consuming, and is unpractical for a large collection of samples. An alternative approach would be to obtain whole genome draft sequences for each bacterial strain and to identify candidate biosurfactant synthesis genes by DNA and protein sequenced-based homologies to infer biosurfactant structures at least to the class level (this is no longer unpractical even for large collections). However, additional investigation would be needed to confirm that the candidate genes were involved in biosurfactant synthesis, and then structural analysis required to confirm the type of biosurfactant produced.

We have therefor taken a different approach to assess the diversity of biosurfactants produced by this group of pseudomonads, and have employed a number of simple foam, emulsion and oil-displacement assays to compare behaviours using cell-free culture supernatants or semi-purified biosurfactant samples (for a list of assays that can be used to characterise biosurfactants see [39, 68]). Clearly other compounds present in the supernatants or samples might interact with the biosurfactant to alter behaviours in some assays, though this would be minimised in analyses of closely-related bacteria. Although we have examined the data from individual assays directly (**Figure 3**), we have found that it is more informative to use a multivariate exploratory statistical method such as hierarchical cluster analysis (HCA) to look at similarities between isolates [15].

– FIGURE 3 TO GO NEAR HERE –

The HCA output is in the form of rectilinear cladograms or star-burst–like constellation diagrams from which groups or clusters of similar isolates can be drawn. Furthermore, it is possible to investigate similarities between assay data by two-way clustering, and preliminary analysis of a test set of strains could be used to determine which assays best differentiate between strains before the entire collection is assessed. In our behavioural analysis of pseudomonad biosurfactants, we have used diesel, mineral oil and vegetable oil–based

displacement assays in which the oil film overlays an aqueous layer of water (pH 6.0) or Tris-buffer (pH 8.0). The resulting constellation diagram separates the twenty-five biosurfactant producing strains and five negative controls into four large clusters, demonstrating that significant biosurfactant chemical and behavioural diversity exists within this collection (**Figure 4**), whilst the two-way clustering information indicates that the three oils differentiates the strains more than the pH of the aqueous layer. If we were to go on to determine the structures of five biosurfactants produced by these pseudomonads, we would choose two strain pairs from the two most distant clusters to maximise the chance of finding different structural classes and different types within classes, e.g. Strains 2 and 3 from the top-right cluster and 6 and 13 from the bottom left cluster shown in **Figure 4**. Alternatively, our choice of candidates for further analysis might also take into consideration particular assay results which might be more relevant for proposed applications, e.g. Strains 7 and 24 show particularly high levels of activity in the oil displacement assay shown in Figure 3 and these might be more interesting for further analysis. It should be noted that choosing within-cluster pairs is risky as the biosurfactants they produce may be identical, and the minor differences shown in the constellation diagram might be due to the presence of other compounds differentially produced by the strains which interfere with biosurfactant behaviours.

– FIGURE 4 TO GO NEAR HERE –

In this analysis of strong biosurfactant-producing pseudomonads and other work [35, 52], we have also been interested in differentiating strains based on phenotype using a HCA approach. A range of simple growth, biochemical and plate-based assays can be used to demonstrate that a collection is diverse and contains few biological replicates (i.e. a strain isolated more than once from the same sample or from two sampling sites close together), and often is more informative than analytical profile index (API)–like testing or 16S rDNA sequence analysis. This data can also be used to select candidate strains for further analysis as discussed above, as well as provide useful information about culture requirements which might be important when considering larger scale production to isolate biosurfactants for testing or analysis.

CONCLUSION

Biosurfactants continue to be of interest in biotechnology, and new compounds are often selected from a relatively small set of biosurfactant-producing bacterial isolates where a key characteristic is surface activity or strength. Using a statistical approach, we have recently demonstrated that there is a limit (γ_{Min}) to biosurfactant strength of approximately 24 $mN.m^{-1}$. We suggest that stronger compounds are not produced by bacteria because of the need to reduce self-harm to the producing cells, though this mechanistic explanation requires experimental confirmation. However, despite a limit to biosurfactant strength, it is clear that even within small collections of bacteria expressing strong biosurfactants that sufficient chemical variation exists to satisfy the need for biosurfactants with different behavioural characteristics suitable for a range of biotechnological applications.

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Figure Legends

Figure 1. **Bacteria produce a range of biosurfactants of varying strengths.** Shown here is a hypothetical distribution of the liquid surface tension reducing abilities of a collection of bacteria producing a range of surface active compounds including biosurfactants. Strains shown ranked from strongest (left) to weakest (right) along the *x*-axis with an arbitrary measure of liquid surface tension on the *y*-axis. The action of a biosurfactant in an aqueous solution such as culture medium is to lower the liquid surface tension (A) (strong biosurfactants show the greatest activity and lower liquid surface tension). Some surface active compounds may show sufficient strength to be considered biosurfactants (black arrows), whilst others may only have a weak effect (grey zone) or act to increase liquid surface tension (grey arrow). However, in any survey of bacteria, there will be one expressing a biosurfactant with the strongest activity (B). It is possible that stronger biosurfactants might be identified in further work, but there must also be a physical limit to the extent to which liquid surface tensions can be reduced and still allow bacterial growth (C). The diversity of strains and biosurfactants will decrease with increasing strength, with relatively few strains producing strong biosurfactants, and potentially one type or group of biosurfactants having the strongest activity.

Figure 2. **Significant variation in biosurfactant strengths are seen in collections of biosurfactant-producing bacteria.** Shown here are the results of quantitative tensiometry of cell-free King's B culture supernatants of a collection of *Pseudomonas* spp. strains ranked in order of surface activity (strength). The dashed line (A) indicates the liquid surface tension of sterile King's B medium, and the solid bar (B) indicates a homogeneous set of twenty-five strong biosurfactant-producing bacteria (identified by *post hoc* Tukey-Kramer HSD, α = 0.05) which are examined in further work (including Table 1 and Fig. 3 here). Means and standard errors are shown. Data are from K. Kabir & A.J. Spiers (unpublished observations) and will be published in full elsewhere.

Figure 3. **Behavioural diversity can be assessed by simple quantitative assays.** Shown here are the results of an oil displacement assay using cell-free culture supernatants and mineral oil overlaid on pure water. Twenty-five *Pseudomonas* spp. strains produce biosurfactants and show considerable variation in the displacement of oil films, whilst the five negative control strains (Controls) show negligible activity. Means and standard errors are shown. Data are from K. Kabir & A.J. Spiers (unpublished observations) and will be published in full elsewhere.

Figure 4. **Significant behavioural diversity exists within the group of biosurfactants with the highest activity.** Behavioural assays can be used to assess the diversity of biosurfactants within a collection in order to identify candidates for further characterisation or testing for a biotechnological application. Shown here is a constellation diagram showing similarities between thirty *Pseudomonas* spp. strains, of which twenty-five express biosurfactants (grey

circles) and five controls which do not (white circles). Similar strains are grouped together, with the four main groups indicated by dashed circles; dissimilar strains are those found the furthest apart on the diagram. This diagram was produced by a hierarchical cluster analysis (HCA) of oil displacement assays testing diesel, mineral and vegetable oil overlaid on water (pH 6.0) or Tris-buffer (pH 8.0) water. The diagram is rooted arbitrarily mid-way along the longest branch (circled dot). In the top left the clustering of the six assays is shown, indicating that behaviours were more similar within than between oils. Data are from K. Kabir & A.J. Spiers (unpublished observations) and will be published in full elsewhere.

Tables –

Table 1. Predicted limit for liquid surface tensions produced by bacterial biosurfactants

Origin of bacteria	Best fitting 3-parameter distribution	*N*	*P*	AD	Predicted limit ($mN.m^{-1}$)	Reference
Contaminated soil and activated sludge	Log-logistic	50	0.294	0.497	24.24	[35]
Soil	Gamma	38	0.233	0.688	24.16	[16]
Soil	Log-normal	25	0.784	0.237	24.74	*
Random sampling of published reports	Weibull	59	0.386	0.238	24.23	[35]

N, Number of bacterial strains samples; *P*, p-value (a large value is required); AD, Anderson-Darling test statistic. Individual distribution identification (IDI) was used to fit theoretical probability distributions to liquid surface tension data, and the minimum liquid surface tension predicted from the threshold parameters. Only the best-fitting distributions are listed. * Data and analyses are from K. Kabir & A.J. Spiers (unpublished observations) and will be published in full elsewhere.

Table 2. Recent reports of bacterial biosurfactant strengths (2015 – 2016)

Bacteria	Liquid surface tension ($mN.m^{-1}$)	Reference
Bacillus spp. strains	28.6 – 60.4	[48]
Bacillus licheniformis R2	28	[22]
Bacillus subtilis M15-10-1	~30	[17]
Corynebacterium xerosis NS5	31.4	[11]
Lactic acid bacteria, various spp.	~55 – 75	[49]
Pseudomonas aeruginosa DSVP2	~30	[60]
Rouxiella sp. DSM 100043	28	[27]
Tsukamurella pseudospumae DSM44118	28.7	[26]
Oil-degrading marine bacteria, various spp.	28.2 – 52.7	[36]
Oil-contaminated soil, various spp.	< 40	[29]
Petrochemical-contaminated soil, various spp.	~50 – 60	[45]

Papers reporting the liquid surface tension of biosurfactants expressed by bacteria were selected using PubMed (on the 28 August 2016) with the key words ‘bacteria’ and ‘surfactant’ and with a publication date range of September 2015 – August 2016. Approximate strengths are those determined from liquid surface tension figures.

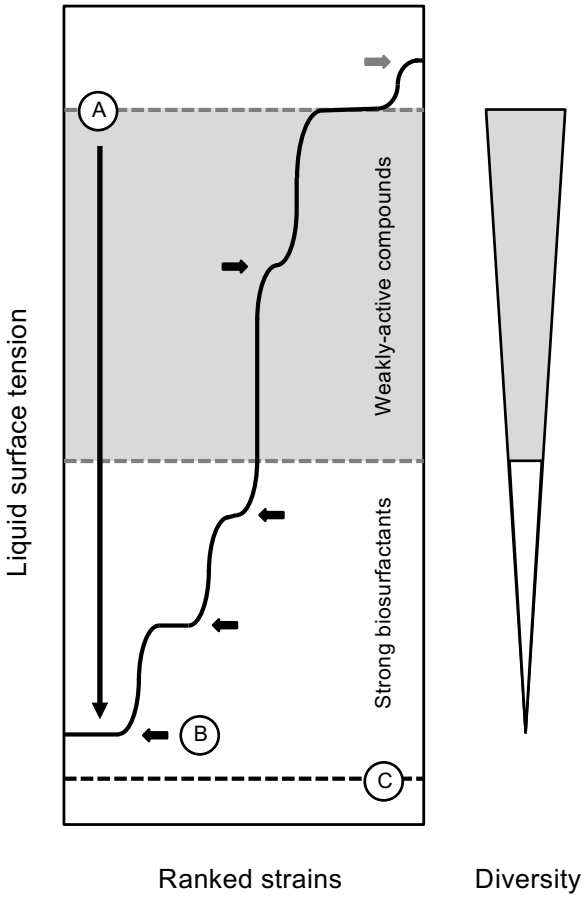
A
B
C
Weakly-active compounds
Strong biosurfactants
Liquid surface tension
Ranked strains
Diversity

Figure 1.

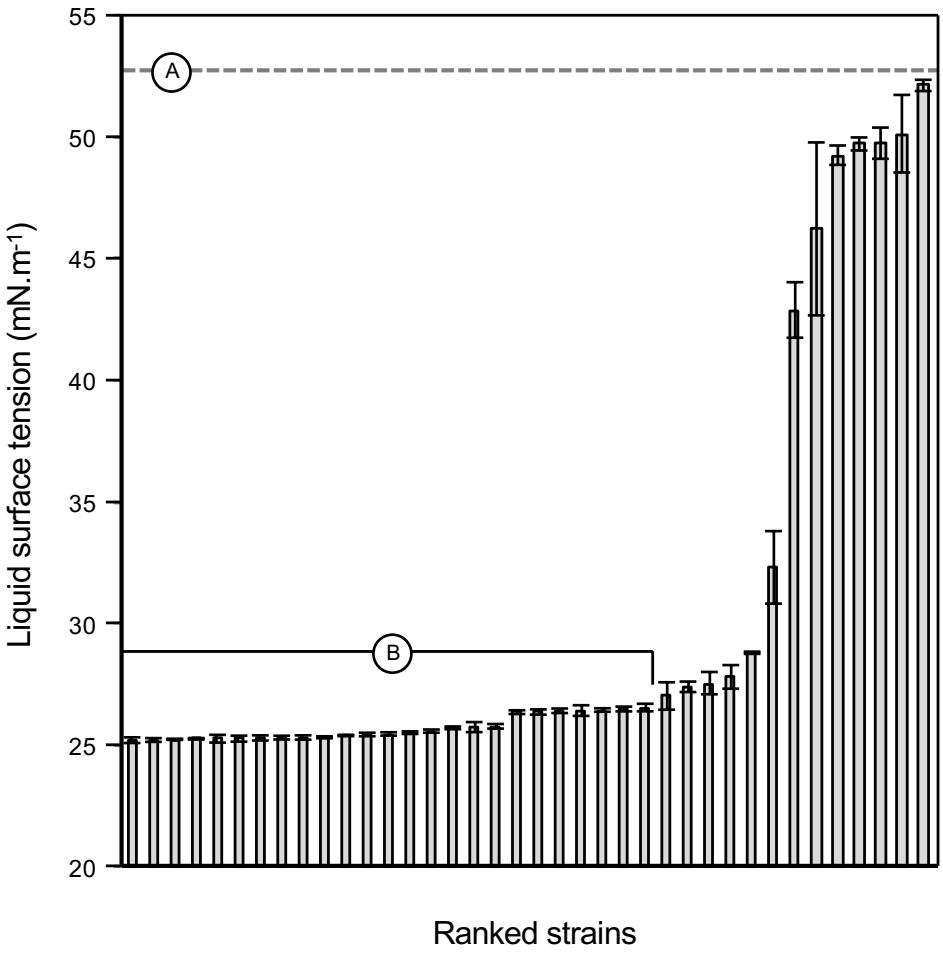
55
50
45
40
35
30
25
20
A
B
Liquid surface tension (mN.m-1)
Ranked strains

Figure 2.

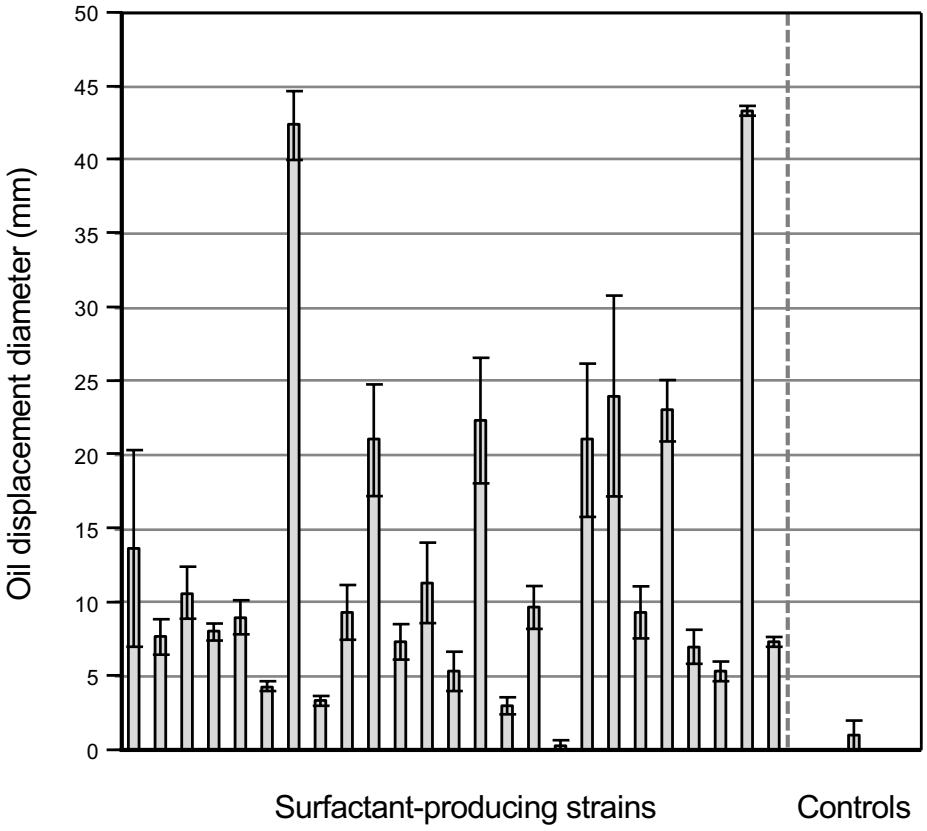
Oil displacement diameter (mm)
50
45
40
35
30
25
20
15
10
5
0
Surfactant-producing strains
Controls

Figure 3.

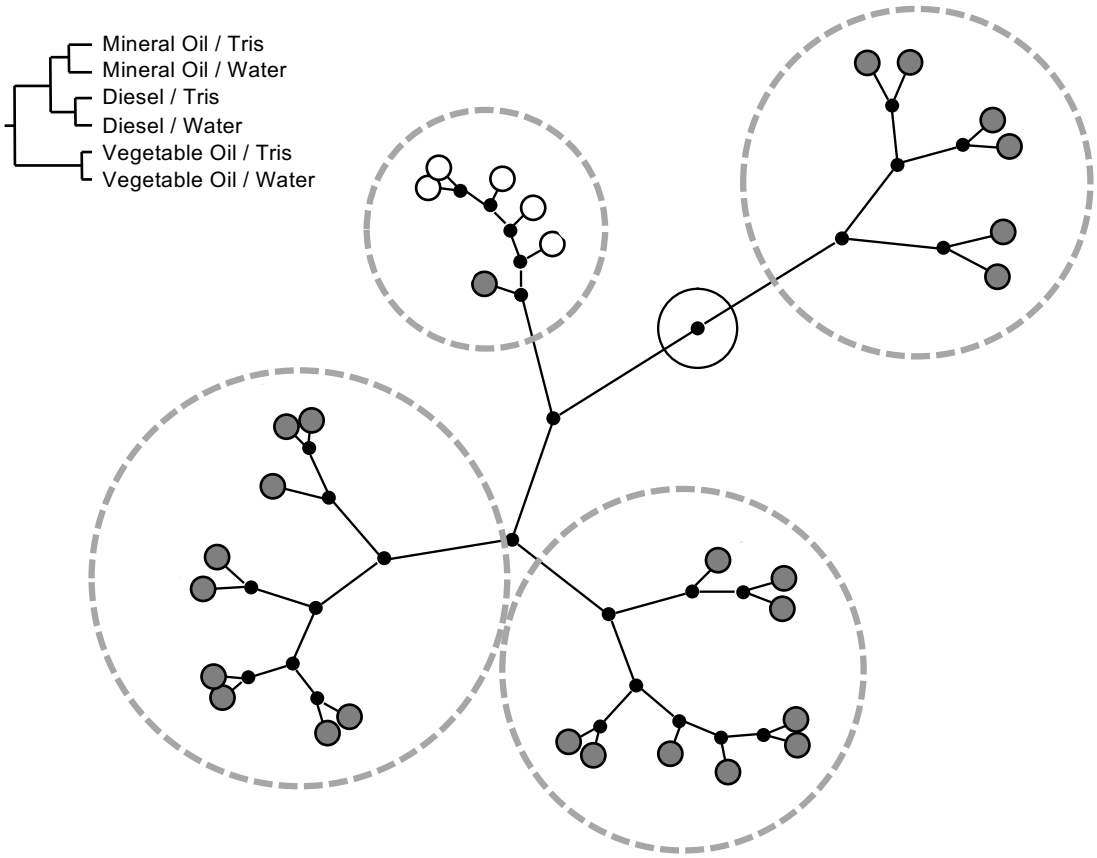
Mineral Oil / Tris
Mineral Oil / Water
Diesel / Tris
Diesel / Water
Vegetable Oil / Tris
Vegetable Oil / Water

Figure 3.