Atmos. Chem. Phys. Discuss.,
doi:10.5194/acp-2016-447-AC2, 2016

Atmospheric Chemistry and Physics Discussions
Open Access
EGU

Interactive comment on "Screening of cloud microorganisms isolated at the puy de Dôme (France) station for the production of biosurfactants" *by* Pascal Renard et al.

Pascal Renard et al.

a-marie.delort@univ-bpclermont.fr

Received and published: 24 August 2016

Dear reviewer 2, Please find below our answer and enclosed our revised manuscript entitled" Screening of cloud microorganisms isolated at the puy de Dôme (France) station for the production of biosurfactants", by P. Renard, I. Canet, M. Sancelme, N. Wirgot, L. Deguillaume, and A.-M. Delort that we would like to publish in Atmospheric Chemistry and Physics; as well as, the supplementary material, the new figures 2 and 4ab, and the answers to the reviewers.

Kind regards

Manuscript acp-2016-447 Screening of cloud microorganisms isolated at the puy de

Printer-friendly version

Discussion paper

Dôme (France) station for the production of biosurfactants P. Renard, I. Canet, M. Sancelme, N. Wirgot, L. Deguillaume, and A.-M. Delort

Answer to Referee #2 First we would like to thank to the reviewers for their work and interest in our work. We have taken into account their comments to improve the manuscript and answered point by point to their questions. Changes in the manuscript are underlined in yellow.

Anonymous Referee #2 English grammar: This paper must be completely proofread by a native English speaker before publication should be considered.

Author's changes The manuscript has been proof read by ACS services

Anonymous Referee #2 -Clouds: the largest hole in this manuscript is the lack of cloud-water analysis. The authors acceptably demonstrate that biosurfactant producing bacteria exist in their cloud water samples but fail to demonstrate if the bacteria actually have a measurable effect on their collected cloud droplets. Even if the authors are unable to measure surface tension depression in their cloud samples, this should still be noted and contextualized in the manuscript. The paragraph in the discussion section, starting P14-L33, would greatly benefit from this analysis.

Author's response We deeply agree with this comment. To demonstrate if the bacteria have a measurable effect on the formation of cloud droplets is the critical issue, and the point of further studies. Nozière et al. (2014) observed strong decreases of surface tension on aerosols: "The first results have shown that these fractions are much more surface-active than expected (σ: 30 mN m-1) and display properties similar to those of biosurfactants such as surfactin or rhamnolipids". Here, we demonstrate that bacteria sampled in clouds are able to produce biosurfactants under lab conditions. We are currently isolating and characterizing these biosurfactants. We have identified 11 different structures by mass spectrometry. In the future we want to collect atmospheric water samples (rain and cloud) and also aerosols and look for these structures in these atmospheric samples (this is what is proposed in the conclusion). This is a long term research plan. We would like to add that, although we did not measure surface tension in cloud waters, we recently measured it in concentrated rain (x300) and found a value of 30 mN/m-1.

Author's changes In order to emphasize this point, we have modified the following sentences: P 11 Line 7: "In the present study, we showed that under laboratory conditions, the most efficient biosurfactant-producing microorganisms (σ < 45 mN m-1) belonged to a limited number of bacterial

genera (Pseudomonas and Xanthomonas) from the ÏŠ-Proteobacteria class (78%) and a yeast genus (Udeniomyces) from the Basidiomycota phylum (11%)." P 13 lines 4: "In conclusion, the results of the present study showed that the microbial strains isolated from cloud waters produce strong biosurfactants under laboratory conditions. The major and most active producers belong to the Pseudomonas genus, which is prevalent in cloud water and typically originates from the phyllosphere. Although the presence of surfactants has been shown on aerosols (Nozière et al., 2014), it has not yet been demonstrated in clouds, and the structure of these compounds has not been established. The biosurfactants overproduced by the best producers in the present study will be isolated to analyze their chemical structure. In parallel, the biosurfactants from cloud aerosols and rain samples will also be extracted, and their structural fingerprints will be analyzed and compared with the signatures of microbial surfactants isolated from clouds."

Anonymous Referee #2 Arbitrary choices: While not debilitating to the paper itself, two arbitrary divisions are made in this manuscript: 1) the surface tension division of Fig.3 . . . For the surface tension divisions, the authors cite Baudel et al. (2012) and Ekstrom et al. (2010) saying that their divisions are chosen in a similar way. However, neither Baudel nor Ekstrom divide their data in the same way that the authors here are trying to do. It would be more correct to say that the authors are choosing bins for their samples that match 1-2 bins of previously published works. The other bins are completely arbitrary and the authors do not provide any reasons for why they chose

>55, 45-55, 30-45, and <30 mN/m. Some reasoning behind these bins needs to be present in the manuscript.

Author's response You are right, Ekström et al. (2010) and Baduel et al. (2012) have not, strictly speaking, defined categories. Our categories rather correspond to the values observed by them. As cited thereafter the value of 30 mN m-1 refers to strong biosurfactants, the value of 45 mN m-1 to HULIS. We agree that the value of 55 mN m-1 is not so well defined and is more arbitrary. Note that our comments in the text greatly refer to values < 45 mN m-1 . Furthermore, we agree, to categorize quantitative variables is always somewhat arbitrary but it helps to present the results. According to Ekström et al. (2010), 30 mN m-1 is the distinct signature of microbial surfactants: "The very low surface tension values obtained with the aerosol samples were attributed to the presence of biosurfactants, because these compounds are the only natural substances able to have such strong effects on the surface tension" "Comparing the curves for the standard compounds with those obtained with the aerosol extracts on Fig. 3 (curves with open circles) clearly show that the latter have the distinct signatures of microbial surfactants: a surface tension below 30 mN m-1 at high concentrations, and a sharp transition characteristic of micelle-forming surfactants". Baduel et al. (2012) also observed strong decrease of surface tension on their atmospheric aerosol samples between 30 and 45 mN m-1): "The minimum surface tension obtained from the summer samples was systematically lower (30 mN m-1) than that of the winter samples (35-45 mN m-1)." According to Ekström et al. (2010), humic-like substances (HULIS) would only lower the surface tension to 45 mN m-1: "This implies that only a few tens of μM of biosurfactants would lower the surface tension of water to about 30 mN m-1. By contrast, 20mM (Ìť 20 g L-1) of HULIS would only lower the surface tension to 45 mN m-1 (Taraniuk et al., 2007), and 10M of malonic acid would lower it to 50 mN/m.". 3 55 mN m-1 is probably the most arbitrary limit; it approximates the first surface tension values measured on aerosols filter samples: "So far, the only way to perform such measurements is with aerosol filter samples, which are extracted, and the surface tension of the extracts measured with a tensiometer. The first studies using these methods reported surface tensions between 52 mN m-1 (Mircea et al., 2005) and 60 mN m-1 (Capel et al., 1990; Facchini et al., 1999, 2000; Hitzenberger et al., 2002; Decesari et al., 2005; Mircea et al., 2005). The small amounts of material on the filters made these methods challenging. An additional drawback was that the extraction, usually in water, was not specific to surfactants, leading to mixtures where the contribution of the surfactants was underestimated." (Baduel et al., 2012).

Author s' changes According to the reviewer remarks we have modified the text as follows: P 9 line 7: we deleted this sentence: "These 4 categories are chosen in a similar way to Baduel et al. (2012) and Ekström et al. (2010)." P 9 line 8: we modified the text as follow: "The first category ($\sigma \leq$ 30 mN m-1) is rare among man-made surfactants and is typical of surfactants of biological origin (Christofi and Ivshina, 2002). In this collection, we observed 34 strains (7%) that reduce the surface tension of the R2A broth below 30 mN m-1. These strains exclusively belonged to the genera Pseudomonas and Xanthomonas (ÏŠ-Proteobacteria, Fig. 1b). The second category corresponded to surface tension values between 30 and 45 mN m-1. The 55 mN m-1 limit is often considered the threshold in terms of the surface tension decrease originating from HULIS (humic like substances) (Kiss et al., 2005; Taraniuk et al., 2007). We observed only 30 strains (6%) in this second category. In summary, from the first two categories ($\sigma \leq$ 45 mN m-1), although new phyla were observed in the second category, the phylum distribution of the most efficient biosurfactant-producing microorganisms remains largely dominated by ÏŠ-Proteobacteria (78% of all strains) and more moderately by Basidiomycota (11%) (Fig. 3). Notably, the two other major taxa of all studied strains, Actinobacteria and α-Proteobacteria, almost completely disappear in these categories. The third and fourth categories (45 < $\sigma \leq$ 55 and σ > 55 mN m-1) represented 28 and 59% of the collection, respectively. The 55 mN m-1 limit is relatively arbitrary but approximates the first surface tension values measured on the aerosol filter samples (Baduel et al., 2012; Capel et al., 1990; Decesari et al., 2005; Facchini et al., 1999, 2000; Hitzenberger et al., 2002; Mircea et al., 2005)."

Anonymous Referee #2 -Arbitrary choices: While not debilitating to the paper itself, two arbitrary divisions are made in this manuscript: [. . .] 2) the source region division of Fig. 4 For the source region divisions, the authors present no reasoning for dividing the air masses into marine/highly marine/etc. From Table S1, it is impossible to tell why marine and highly marine are split. Fig. S1 suggests the split is because of time over open ocean but the authors need to be explicit here. I would also argue that Fig. 4 adds nothing to the manuscript and should be replaced with the HYSPLIT trajectories and some additional meteorological statistics (e.g. wind direction histogram for the sampling site for both cloud-sampling days and non-sampling days). There is also no reason to not show the air mass height results from HYSPLIT.

Author's response We understand from the reviewer's remarks that our text was not clear and did not give enough details to be understandable. The different categories as described in Figure 4 are based on the paper of Deguillaume et al. (2014). In this paper the air masses are defined according two different types of criteria: 1) Their back trajectories : They are calculated using the HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) model with the GDAS1 meteorological data archive and default settings (Draxler and Rolph, 2010). Considering 10 years of monitoring at the puy de Dôme station, Deguillaume et al (2014) divided France in four sectors to classify these back trajectories crossing France (West, North East, NorthWest/North, South-West/West). 2) The chemical content of cloud water: The physicochemical parameters presented in Table S1 (pH, SO42-, NO3-, Cl-, Acetate, Formate, Oxalate, Succinate, Malonate, Na+, NH4+, Mg2+, K+,Ca2+) are used to make an ACP analysis as described in Deguillaume et al. (2014). This ACP gives 4 different groups which have been named “highly marine”, “marine”, “continental” and “polluted”. Typically, the more “polluted” are the clouds, the lower is the pH and the higher are the concentrations of NH4+, NO3-, SO42-. The more “marine” are the clouds, the higher is the concentration of NaCl. In our opinion the second type of categories based of chemical measurements is more accurate as it reflects the whole history of the clouds integrating their eventual complex trajectories.

Author's changes Therefore, in the revised manuscript we deleted all the data linked to the back trajectories and only kept the "highly marine", "marine", "continental" and "polluted" categories. This includes deletion of "W, NE, NW, NW/N and SW/W" in Tables S1 and S2, of Table S3, of Figure S1, of Figure 4, of Figure 5(a) and Figure 6 (a). Figures 5b and Figure 6b are now Figures 4(a) and (b) in the revised manuscript. P 10, line 11, this text was deleted: "Cloud events are first classified according to the air mass origin - i.e., west (W), north-west/north (NW/N), north-east (NE) and south-west/south (SW/S) - determined from backward trajectories (see Fig. 4 and Fig. S1 in the supplement). Second, cloud events are classified according to the physicochemical characteristics of cloud waters (Marine, Highly marine, Continental and Polluted, see Fig S1 and Table S1 in the supplement) as described by Deguillaume et al. (2014). Figure 4 shows that 18 events come from W consisting of 2 Highly marine, 14 Marine and 2 Continental cloud events, 13 events from NW/N with 10 marine, 2 continental and 1 polluted cloud events, 3 events from NE with 2 polluted cloud events and the other continental and, from the SW/S, 5 events of which 2 marine and 3 continental cloud events." P 11 line 10, this text was deleted: Figure 5a shows the distribution of surface tensions values (σ) measured from the 480 strains tested for biosurfactant production, according to the air mass origins (4 sectors: W, NW/N, NE and SW/S). Samples from west sector constitute the great majority of our collection (318/480 strains). From statistical analysis, we observe a significant difference (Kruskal-Wallis p-value: 0.0049 « 0.05) in the distribution of biosurfactant-producing microorganisms between the NW/N sectors and the others (W, NE and SW/S). The Mann-Whitney test (see details in the Supplement, Table S3) allows us to attribute this difference to the NW/N sector with a surface tension median (53 mN m-1) significantly lower (Mann-Whitney p-values < 0.05) than the other three sectors (medians: 59, 60 and 61 mN m-1, for W, NE and SW/S sectors, respectively). This difference cannot be completely attributed to the differences in the phyla distribution within the different air masses (Fig. 6). Indeed, as shown before, the most efficient biosurfactant-producing microorganisms belong to ÏŠ-Proteobacteria class, which represents 23% of all strains, but its distribution regarding the air mass origin sectors remains unselective (26, 28, 3 and 14% for W, NW/N, NE and SW/S sectors, respectively). The difference in the distribution of biosurfactant-

producing microorganisms between the four sectors is rather due to the proportion of the most efficient biosurfactant-producing microorganisms ($\sigma \leq$ 45 mN m-1) amongst the ÏŠ-Proteobacteria class (Fig. 6). In the NW/N sector, most efficient biosurfactant-producing microorganisms account for 68% of ÏŠ-Proteobacteria (13/19 isolates), against 40% in the other three sectors (37/93 isolates). No such difference amongst the ÏŠ-Proteobacteria class is observed in the chemical composition groups. P 10 line 6, We have added these sentences in the modified manuscript: "The main parameters, including pH, SO42-, NO3-, Cl-, acetate, formate, oxalate, succinate, malonate, Na+, NH4+, Mg2+, K+, and Ca2+, are summarized in the Supplemental materials (Table S1). These physico-chemical parameters were used for the ACP analysis as described in Deguillaume et al. (2014). The ACP generated 4 different types of clouds, classified as “highly marine”, “marine”, “continental” and “polluted”. Typically, the more “polluted” clouds have a lower pH and higher concentrations of NH4+, NO3-, and SO42-. The more “marine” clouds have a higher concentration of NaCl. The 39 cloud events were divided into 2 “highly marine”, 26 “marine”, 8 “continental” and 3 “polluted” clouds (Table S1)."

Anonymous Referee #2 Furthermore, the analysis starting on page 11, which attributes statistical difference between the air mass divisions (one of which has 3 samples!), seems weak. I would suggest that there are stronger ways to segregate the air masses given the Table S1. I believe the whole analysis should be rerun using the chemical speciation data. Shorter Comments: -Mann-Whitney and KW ANOVA – can the authors comment on how appropriate it is to assume statistical independence for air masses that, though they are measured at the sampling site as from 2 different directions, shared the same path as few as 5 days prior to sampling? -Figure 5 needs to be replaced with a proper box and whiskers plot or some other way to judge what the real spread in the data looks like (possibly note the std. dev.?)

Author's response As explained above, Deguillaume et al (2014) classifies clouds according to two different criteria: back trajectories and physico-chemical parameters. In our opinion the second type of categories based of chemical measurements is more accurate as it reflects the whole history of the clouds integrating their eventual complex trajectories. Therefore, in the revised manuscript we have deleted all the data and text linked to the back trajectories and only kept the "highly marine", "marine", "continental" and "polluted" categories. Our statistical analyses are based on 39 cloud events with 480 different strains. This represents, to our knowledge, the largest data set on cloud samples ever studied; it is representative of cloud sampling over more than 10 years at the puy de Dôme station. These cloud events when classified according to ACP analysis (Deguillaume et al. 2014) are independent and can be compared. The only problem is that it is still difficult to make statistics on samples with such intra- and inter-sample variations (not because there is only 3 samples for example). For example, in marine clouds, we identified only one strain in few events (e.g., event 29) compared to the 62 strains in the event 54 (see Table S1 in supplementary).This makes our Mann-Whitney and Kruskal-Wallis tests a bit weak. We have therefore concluded it would be better to be limited to a high-quality observation rather than making questionable statistics, which would not adding much new, i.e., the correlation of Pseudomonas / surface tension. In conclusion we decided to keep the paragraph "Impact of the origin and chemical composition of clouds on biosurfactant production" to give some general tendency. The obtained results are interesting as they suggest a link between the vegetation origin and the biosurfactant production. This should be studied in more details in the future.

Author's changes In the abstract, we replaced: Statistical analyses showed some positive correlations between the origin of air masses and chemical composition of cloud waters with the presence of biosurfactant-producing microorganisms, suggesting a "biogeography" of this production. by: We observed some correlations between the chemical composition of cloud water and the presence of biosurfactant-producing microorganisms, suggesting the "biogeography" of this production. Page 4 line 5: we replaced: “In order to evaluate the potential correlation between the origin of air masses and composition of cloud waters and the presence of biosurfactant-producing microorganisms, statistical analyses are performed.” by: "We observed a potential correlation between the composition of cloud waters and the presence of biosurfactant-producing microorganisms." P5 line 14: This text has been deleted: 2.4 Statistical analyses Herein, we investigate the differences, in terms of impact on the non-normally distributed surface tension, due to the origin of air mass and the chemical composition of clouds using the PAST software version 3.09 (Hammer et al., 2001). Using a non-parametric method, the Kruskal-Wallis one-way analysis of variance (Siegel, 1956), we compare the distributions of surface tensions between 4 air mass origin sectors: west (W), north-west/north (NW/N), north-east (NE) and south-west/south (SW/S) and between 4 chemical composition groups (Marine, Highly marine, Continental and Polluted). P-value < 0.05 is considered statistically significant. Mann-Whitney test (Mann and Whitney, 1947), which is a measure of how different two populations are, allows specifying which group dominates, with two-by-two comparison. Page 10 line 1: we totally rewrote the section 3.3 and replaced by: 3.3 Potential impact of the chemical composition of the clouds on biosurfactant production In the present study, the screened microbial strains were isolated from 39 cloud events presenting different profiles. Information on the cloud chemical composition and the physicochemical parameters measured at the puy de Dôme station and described in (Deguillaume et al., 2014) is provided on the website of the Observatory of Earth Physics in Clermont-Ferrand (http://wwwobs.univ-bpclermont.fr/SO/beam/data.php). The main parameters, including pH, SO42-, NO3-, Cl-, acetate, formate, oxalate, succinate, malonate, Na+, NH4+, Mg2+, K+, and Ca2+, are summarized in the Supplemental materials (Table S1). These physico-chemical parameters were used for the ACP analysis as described in Deguillaume et al. (2014). The ACP generated 4 different types of clouds, classified as “highly marine”, “marine”, “continental” and “polluted”. Typically, the more “polluted” clouds have a lower pH and higher concentrations of NH4+, NO3-, and SO42-. The more “marine” clouds have a higher concentration of NaCl. The 39 cloud events were divided into 2 “highly marine”, 26 “marine”, 8 “continental” and 3 “polluted” clouds (Table S1).

Figure 4 (a). Surface tension (σ) distribution of the 480 strains examined for biosurfactant production according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted). Highlighted in blue, the number of tested strains. Box and whisker plots are shown with the minimal (red) and maximal (green) surface tensions. The orange boxes represent the 25th and 75th percentiles of the measurements (b). Phyla distribution according to the physicochemical characteristics of the cloud waters (marine, highly marine, continental and polluted). Figure 4a shows the distribution of the surface tensions values (σ) measured from the 480 strains examined for biosurfactant production according to the cloud water chemical composition (marine, highly marine, continental or polluted). A comparison of the distribution of the phyla of the strains in the same cloud events is presented in Figure 4b. The samples from marine clouds constitute the majority of this collection (323/480 strains). We observed a difference between the surface tension values from continental and highly marine strains (medians: 56 and 61 mN m-1, respectively). Highly marine clouds are characterized by the highest minimal surface tension (45 mN m-1, Figure 4a), consistent with the almost complete absence of ÏŠ-Proteobacteria, which are the most efficient biosurfactant-producing microorganisms ($\sigma \leq$ 45 mN m-1) (1/57 isolates, see Figure 4b). These observations were based on 39 cloud events with 480 different strains, representing, to our knowledge, the largest cloud sample data set studied; this data set is representative of cloud sampling over more than 10 years at the puy de Dôme station. Although it remains difficult to generate statistics on samples with such intra- and inter-sample variations, these results provide a general tendency that could be reinforced and confirmed with more data in the future. Figure 4, Figure 5a, Figure 6a, Table S3 and Figure S1 have been deleted. We have kept only Figure 5b and Figure 6b which are now Figure 4(a) and (b) in the revised manuscript, note that the presentation of the data has been modified as suggested by the referee (whiskers plots).

Anonymous Referee #2 Paragraph starting P15, L26 – I am generally a proponent of contextualizing your work but I am not convinced this paragraph adds anything to the manuscript. It should either be removed or condensed and moved to the introduction.

Author's response We agree with the referee that this paragraph is a bit out of scope of this paper, therefore it has been deleted.

Author's changes Page 15 line 26, this paragraph has been deleted: To our knowledge, research on the potential impact of biosurfactants on human health due to their presence in atmospheric waters remains marginal, compared to terrestrial or aquatic ecosystem studies (Olkowska et al., 2014). Aerosols are now well-known to represent a major concern for the populations as shown by epidemiology studies (Bernstein et al., 2004; Dominici et al., 2014; Pope, 2000). The toxicological impact is inversely proportional to the particle size (Nel, 2005; Novák et al., 2014). For example, fine particles (PM1-2.5) reach lung alveoli and ultrafine particles (PM0.1), once in the lung, would readily pass into the bloodstream and cause a direct insult to the cardiovascular system and other organs (Moshammer and Neuberger, 2003; Polichetti et al., 2009). The composition of the aerosols is also of major importance and should not be underestimated (Brimblecombe and Latif, 2004; Škarek et al., 2007). Regarding more precisely surface-active organic aerosols, few reports are devoted to synthetic molecules. Thus, Poulsen et al. (2000) suggested that molecules with surfactant properties could interfere in the immunological pathways, which could explain the increase of allergic diseases in industrialized societies. At high concentration in the atmosphere, surfactants can lead to asthma, can disrupt the stability of human respiratory systems, as well as can cause dry eyes allergies (Ahmad et al., 2009; Xinxin et al., 2016). Rhamnolipids inhibit ciliary function and produce damage to the human bronchial epithelium (Abdel-Mawgoud et al., 2011). Surfactants, by lowering the surface tension of tear films of the eyes could also be at the origin of dry eyes sensation (Vejrup and Wolkoff, 2002). Hence, biosurfactants present on aerosols could have a double impact on human cells, first because they could destroy cell membranes of the host, second because they could concentrate and dissolve toxic pollutants and help their penetration within the host cells. Further studies are needed to better evaluate the impact of surfactant on human health.

Anonymous Referee #2 Minor comments: - S2.2, P4, L23-25 : Given that most of the people reading ACP are not biologists, a one sentence explanation on why those three specific agars were chosen should be added to the text. Also, TSA should be defined. -P5, L11: Should read “3.2” not “III.2”

Author’s changes - Page 4 line 18, the text has been modified as follows: "Triplicate volumes of 0.1 mL of cloud water were plated onto R2A agar growth medium (Reasoner and Geldreich, 1985; DIFCOTM), and eventually onto R2A medium supplemented with NaCl 20 g L-1 and King’s B (King et al., 1954), Sabouraud (DIFCOTM) and TSA (Trycase Soy Agar, DIFCOTM) media. The plates were incubated at 17°C or 5°C under aerobic-dark conditions until the appearance of colonies (typically 6 days at 17°C or 10 days at 5°C) (Vaïtilingom et al., 2012). R2A medium is a poor medium initially developed to isolate microorganisms from tap water and is well adapted to cloud samples, which are also poor. The addition of NaCl to R2A favors the selection of marine microorganisms; King’s B medium is selective for Pseudomonas strains, while Sabouraud medium is selective for yeast strains." - Page 5, Line 1, Should read “3.2” not “III.2”: Done

Please also note the supplement to this comment:
http://www.atmos-chem-phys-discuss.net/acp-2016-447/acp-2016-447-AC2-supplement.pdf

Interactive comment on Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2016-447, 2016.

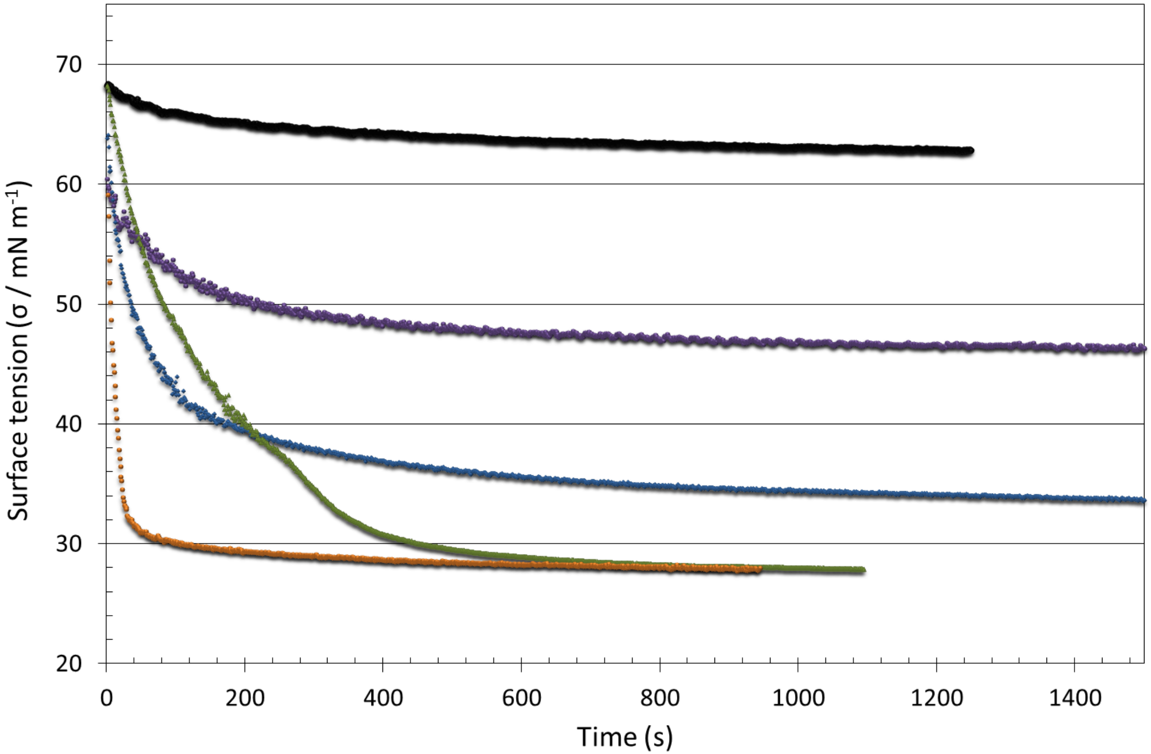

Figure 2. Time profile of surface tension measurements in a non-logarithmic form.

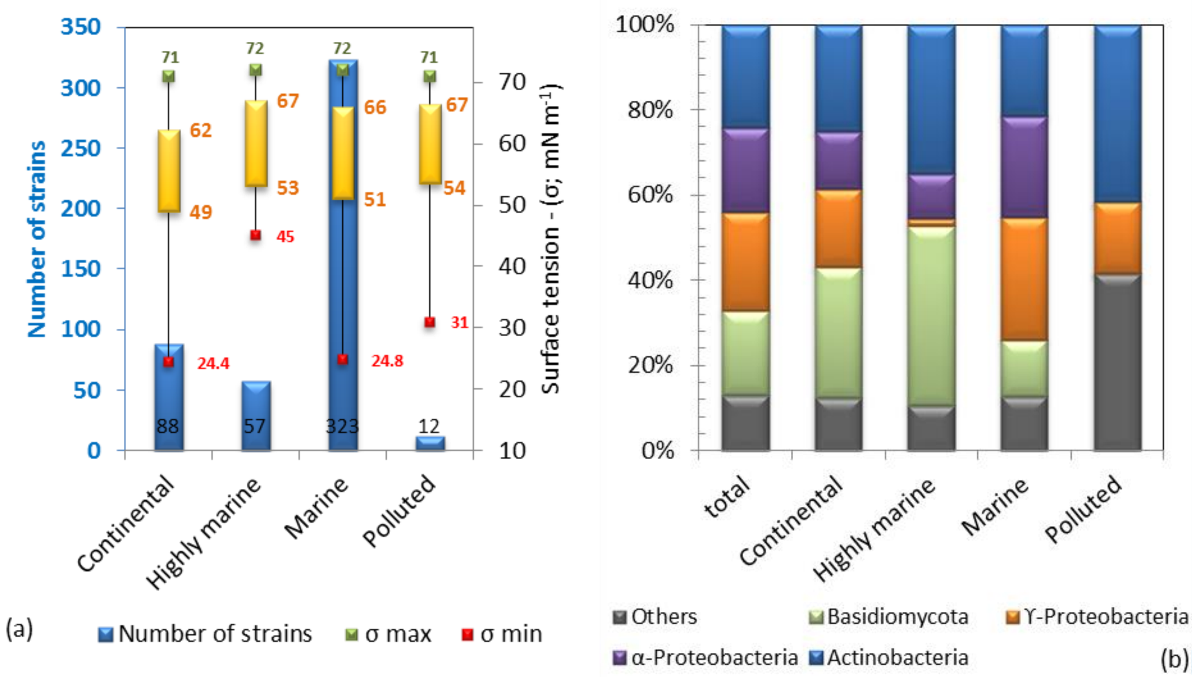

Figure 4 (a). Surface tension (σ) distribution of the 480 strains tested for biosurfactant production according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted). Highlighted in blue, the number of tested strains. Box and whisker plots are shown with the minimal (red) and maximal (green) surface tensions. The orange boxes represent 25th and 75th percentiles (lower and upper quartiles) of the measure **(b).** Phyla distribution according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted).