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

Dear reviewer 1,

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 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 #1 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 #1 The authors report equilibrium surface tension values for every microorganism in their study. However, it is unclear how they determine when equilibrium has been reached. All of the surface tension time profiles in Figure 2 appear to be decreasing when the measurements were stopped. That is, the reported equilibrium surface tension values are the minimum values for the time profiles given, but may not be if the time profile was extended. In section 2.3, the authors state a 30-minute maximum for surface tension measurements but give no justification for this time frame.

Author's response You are absolutely right, the minimum of the equilibrium region (σeq), is difficult to determine experimentally (small variations of surface tension over long timescales) (Nozière et al., 2014). The surface tension decreases asymptotically and the logarithmic scale of the figure 2 is probably misleading, it looks clearer when presented in a non-logarithmic form (see enclosed Figure 2. Time profile of surface tension measurements in a non-logarithmic form.). The overall equilibration time, teq, is of the order of $2 \times$ tm (time of meso-equilibrium) (Nozière et al., 2014). After this period, surface tension decreases marginally. According to the measurements we did on longer period (few hours), our overestimation is comprised between 0.1 and 0.2 mN m-1. That is why, above 30 mN m-1, we only give nominal surface tension (Table S2 in the supplementary). Below 30 mN m-1 and above the CMC, surface tension decreases quickly and it is easier to be more accurate. Finally, we decided to keep our original Figure 2 presented in the logarithmic mode as it is the usual way in publications and allows to show the initial surface tension (σ0), the surface tensions in the

meso-equilibrium (σm) and in the equilibrium (σeq) phase (See Noziere et al 2014 for instance).

Anonymous Referee #1 In Figure 2, the authors show the surface tension time profile for the R2A broth, which is the medium used for all cultures of their isolated microorganisms. However, this may not be a good baseline because incubation period lasts between six to ten days. We accept that the microorganisms are altering the composition of the broth by producing biosurfactants, but they are consuming nutrients in the broth as well. It remains to address how the removal of nutrients would impact the surface tension of the crude extracts.

Author's response We agree that broth, i.e. carbon sources, influences biosurfactant production. This is true for industrial production of biosurfactants performed in aqueous media with a rich carbon source feedstock, such as carbohydrates, hydrocarbons, fats, and oils. Such enriched broths increase the production. However in our case R2A broth is very poor so when the microorganisms consume the carbon sources, it does not make a great change. We confirmed this point thanks to the following experiments: We did purification of few biosurfactants from microbial R2A cultures, and then we measured the surface tension of these pure compounds both in water and in R2A medium, the differences were marginal (confidential, to be published).

Author's changes We modified the text as follows: P 9 line 9: In this collection, we observed 34 strains (7%) that reduce the surface tension of the R2A broth below 30 mN m-1.

Anonymous Referee #1 Furthermore, the authors present a large amount of data regarding the surface tension of crude extracts but do not make a connection to the surface tension of cloud water, which is arguably the basis for this work. Since the authors have already collected the cloud water in order to isolate the microorganisms, it would be useful to also report surface tension values for the cloud water samples as well extend the crude extract results to cloud water.

Author's response We share your viewpoint; the surface tension of cloud water could have been relevant. Unfortunately, the cloud samplings have been performed before the acquisition of the tensiometer. However, according to the Köhler theory, the surface tension, as well as, the saturation vapor pressure and the CCN diameter, drive the activation of particles into cloud droplets. The activation occurs when the radius of the cloud droplet is minimal (few nm, i.e., wet aerosol) and the concentration of organic compounds, such as biosurfactant, is maximal (Nozière et al., 2014). The effect of surface tension is maximal during the activation. In cloud droplet (few μm), organic compounds are diluted, and biosurfactants are likely under the CMC. Nevertheless, measuring surface tension in concentrated cloud water could be a complementary work, especially since we observed in 300 fold-concentrated rain, a strong decrease of the surface tension (30 mN m-1) (unpublished data). 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 cloud and rain samples 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.

Author's changes In order to emphasize this point, we have modified the following sentences: 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 #1 Finally, the statistical analysis section did not seem to add much to the paper. The main takeaway was that α-Proteobacteria are efficient biosurfactant producers, which reinforces conclusions from section 3.2. However, the entire analysis seems unsubstantiated. The distinction between air mass origins seems arbitrary. The distinction between chemical compositions is more logical, but the conclusions for that analysis are weaker.

Author's response Our statistics are based on 480 strains but these strains are grouped into 39 cloud events, thus partially dependent. This sampling was spread over 10 years, and represented with related analyzes, a considerable work and a more than correct observation of Puy de Dôme clouds. However it is still difficult to make statistics on samples with such intra- and inter-sample variations. 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 could use mixed model. Nevertheless, you are right, these statistics would not add much new, i.e., the correlation of Pseudomonas / surface tension. We therefore concluded it would be better to be limited to a high-quality observation.

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 re-

placed: "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, SO_4^{2-}, NO_3^-, Cl^-, acetate, formate, oxalate, succinate, malonate, Na^+, NH_4^+, Mg^{2+}, K^+, and Ca^{2+}, 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 NH_4^+, NO_3^-, and SO_4^{2-}. 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 referee 2.

C7

Anonymous Referee #1 The grammatical errors are too numerous to list individually. This paper would greatly benefit from editing by a native English speaker. Authors: The manuscript has been proof read by ACS services Page 5, line 2. Might be helpful to keep units consistent with Page 4, line 33. Either g (preferred) or rpm. Authors: We put these values: 10,480 g / 3 min Page 5, line 11. Change section number from Roman to Arabic numerals. Authors: done Page 9, lines 9-10. This is a misrepresentation because the biosurfactants are reducing the surface tension of the R2A broth, not pure water. Authors: Changed Page 9, line 11. I think you mean surface tension values between 30 and 45 mN m-1 not up to 45 mN m-1. Authors: Yes, we agree, we have changed it to "between 30 and 45 mN m-1" Page 9, line 18. Third and fourth is clearer than third and last. Authors: Changed Page 11, lines 12-14. There is not a significant difference between all four sectors, just between NW/N and the others, according to your supplementary information. Authors: Actually we have deleted all the data linked to the back-trajectories of the air masses (see answer to referee 2) Page 14, lines 3-5. Citation for this sentence? Authors: we have added "(Joly et al., 2015 and references hereinafter)".

Please also note the supplement to this comment:
http://www.atmos-chem-phys-discuss.net/acp-2016-447/acp-2016-447-AC1-supplement.pdf
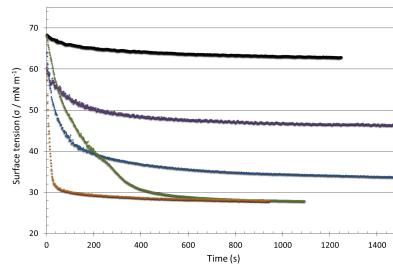
C8

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

Fig. 1. Figure 2. Time profile of surface tension measurements in a non-logarithmic form.
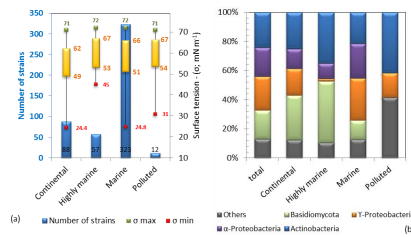
C9

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).

Fig. 2. 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, continent

C10