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To Prof. V. Faye McNeill
Associate Editor, Atmospheric Chemistry and Physics

New submission of manuscript acp-2016-447

Dear editor,

Please find below 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.

We would like to thank all the reviewers for their interest in our work and for all their remarks that greatly helped to improve the manuscript.

We have taken into account all the remarks of reviewers and changed the text accordingly. The corrections are underlined in yellow in the revised manuscript.

The main point was concerning the statistical analysis used to correlate the origin of air masses, considering their back trajectories and their chemical composition with the distribution of the microbial phyla in these air masses.

We agree with the referee that using back trajectories is not the best way to define the origin of clouds, so we have deleted all information related to this point (Figure 4, Figure 5a and Figure 6b were deleted). We only kept the categories defined from ACP analysis taking into account the physicochemical compositions of the cloud samples (highly marine, marine, continental, polluted) as described in Deguillaume et al (2014) (see new the Figure 4 and b, previously as Figure 5b and 6b).

Our statistical analyses are based on 39 cloud events sampled over more than 10 years with 480 different strains; this is the largest data set on cloud samples ever studied. However it is still difficult to make

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statistics on samples with such intra- and inter-sample variations, therefore we have chosen to delete all the statistical data, rather keeping our results as simple observations.

We hope this revised paper will be publishable as an article in *Atmospheric Chemistry and Physics.*

Yours sincerely,

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Screening of cloud microorganisms isolated at the puy de Dôme (France) station for the production of biosurfactants

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Abstract. A total of 480 microorganisms collected from 39 clouds sampled at the puy de Dôme station (alt. 1465 m, 45°46'19" N, 2°57'52" E, Massif Central, France) were isolated and identified. This unique collection was screened for biosurfactant (surfactants of microbial origin) production by measuring the surface tension (σ) of the crude extracts, comprising the supernatants of the pure cultures, using the pendant drop technique. The results showed that 41% of the tested strains were active producers ($\sigma < 55$ mN m^{-1}), 7% being extremely active ($\sigma < 30$ mN m^{-1}). The most efficient biosurfactant producers ($\sigma < 45$ mN m^{-1}) belong to a few bacterial genera (Pseudomonas and Xanthomonas) from the ϒ-Proteobacteria class (78%) and a yeast genus (Udeniomyces) from the Basidiomycota phylum (11%). Some Bacillus strains from the Firmicutes phylum were also active but represented a small fraction of the collected population. Strains from the Actinobacteria phylum in the collection examined in the present study showed moderate biosurfactant production ($45 < \sigma < 55$ mN m^{-1}). Pseudomonas (ϒ-Proteobacteria), the most frequently detected genus in clouds, with some species issued from the phyllosphere, was the dominant group for the production of biosurfactants. We observed some correlations between the chemical composition of cloud water and the presence of biosurfactant-producing microorganisms, suggesting the "biogeography" of this production. Moreover, the potential impact of the production of biosurfactants by cloud microorganisms on atmospheric processes and human health is discussed.

Keywords: Biosurfactants, cloud condensation nuclei, microorganisms

1 Introduction

Atmospheric aerosol particles act as cloud condensation nuclei (CCN) upon which liquid droplets can form. More aerosols increase the concentration of smaller droplets, leading to a brighter cloud (Twomey effect). However, owing to their complexity, all aerosol–cloud–interactions (ACI) can amplify or dampen this effect. Therefore, ACI, particularly CCN activation, still account for major uncertainties in global climate and future climate change predictions (Boucher et al., 2013).

Among organic aerosols, water soluble organic compounds (WSOC) represent a significant fraction of the tropospheric aerosol mass (Kanakidou et al., 2005; Murphy et al., 2006; Saxena and Hildemann, 1996; Zenchelsky and Youssefi, 1979; Zhang et al., 2007) and constitute a complex mixture of neutral and acidic polar organic compounds (Decesari et al., 2001). As some WSOC are amphipathic compounds, these aerosols can act as surfactants (surface-active agents) by creating a partition between the droplet gas-liquid interface and the bulk volume. Seidl and Hänel (1983) were among the first to estimate concentrations of surface-active soluble substances on rainwater and wet aerosol by measuring the lowering of the surface tension. Capel et al. (1990) correlated the surface tension of fog samples with the dissolved organic carbon content. Hitzenberger et al. (2002) observed slight reductions in the surface tension for most of the 23 cloud water samples collected in mountainous and sparsely populated areas.

Some groups (Decesari et al., 2005; Facchini et al., 1999) have renewed research on surface-active compounds in the atmosphere with a great deal of speculation about the potential impact of these compounds on the climate (Brimblecombe and Latif, 2004). Indeed, surfactants affect cloud droplet growth in two main ways according to the Köhler equation (Köhler, 1936): by increasing soluble mass ("Raoult term") and by decreasing cloud droplet surface tension ("Kelvin term") (Decesari et al., 2003; Facchini et al., 1999; Lance et al., 2004; Mircea et al., 2002; Rodhe, 1999; Shulman et al., 1996). Thus, considering both the solute concentration increase and the surface tension decrease, Mircea et al. (2002) calculated a substantial attenuation of the aerosol critical supersaturation, revealing a significant increase in the CCN number concentration. By adding surfactant to the gas-aerosol interface, Sareen et al. (2013) assessed significant enhancements in CCN activity (up to 7.5% reduction in critical dry diameter for activation), which for ambient aerosol would lead to a 10% increase in the cloud droplet number concentration. Facchini et al. (1999) estimated that a population rise resulting from surfactants might result, in all stratus clouds, in a 1% increase in albedo and, subsequently, in a calculated global radiative forcing of -1 W m^{-2}.

In addition to the impact of surfactants on CCN activity *via* both Raoult and Kelvin terms, a third effect should be considered. Surfactants can either enhance or slow down the transfer of water across the surface according to the hydrophilic or hydrophobic nature of this aerosol organic coating (Aumann and Tabazadeh, 2008; Chakraborty and Zachariah, 2011; Feingold and Chuang, 2002; Rudich, 2003). These organic coats are common on aerosol particles and might retard the evaporation of molecules present in the water phase, reduce gas transfer, influence chemical reactions, and alter absorption or reflection properties of aerosols (Clifford et al., 2007; Decesari et al., 2003; Gill et al., 1983; Gilman and Vaida, 2006). Nenes et al. (2002) examined the sensitivity of the cloud droplet number concentration to different chemical factors as the dissolution of soluble gases and solutes or formation of organic films at the droplet surface, demonstrating that these chemical effects on droplet activation could be significant. Similarly, the $^{\bullet}$OH heterogeneous reactions that occur on organics in the troposphere can significantly modify the hygroscopic properties and CCN ability of these organic surfaces (Bertram et al., 2001; Ellison et al., 1999), thus potentially playing an important role in the Earth's radiative balance by affecting the properties of clouds, *e.g.*, Twomey effect and cloud life time (Aumann et al., 2010; Nenes et al., 2002; Rodhe, 1999).

Recent experiments have focused on CCN enhancement resulting from biogenic influence (Facchini et al., 2000; O'Dowd et al., 2002; Svenningsson et al., 2006). Thus, some atmospheric organic compounds, such as pinic and

pinonic acids (produced by the oxidation of terpenes in organic vapors released from the canopy) have been implicated in the reduction of the surface tension of water, even at extremely low concentrations (Li et al., 2010; O'Dowd et al., 2002). Another important class of hydrophobic WSOC is humic-like substances (HULIS). These complex mixtures of high molecular weight compounds can depress surface tension in fog water samples by 15-20% (Decesari et al., 2003; Dinar et al., 2006; Facchini et al., 2000).

Nevertheless, although a number of organic surface-active compounds have been detected in aerosol particles and cloud droplets, a large fraction of WSOC remains poorly characterized (Herckes et al., 2013). Moreover, due to their limited concentrations in aerosols, it remains unknown whether atmospheric organic surfactants decrease the surface tension of atmospheric water or contribute to the CCN properties of atmospheric particles (McFiggans et al., 2006).

In the last decade, a few studies have identified strong organic surfactants in atmospheric aerosols (Baduel et al., 2012). Their exceptional tension-active properties suggested that these compounds could be "biosurfactants" of microbial origin that could affect cloud formation (Ekström et al., 2010; Nozière et al., 2014). Biosurfactants are secondary metabolites produced by microorganisms, including low molecular biosurfactants (mainly glycolipids and lipopeptides) and high molecular mass biosurfactants (polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex mixtures of these polymers) (Gautam and Tyagi, 2006; Rosenberg and Ron, 1999). These amphiphilic compounds reduce the surface tension of atmospheric water below 30 mN m^{-1} (*i.e.*, > -40% compared with pure water) and at concentrations 5 or 6 orders of magnitude lower than organic acids (Ekström et al., 2010). In comparison, HULIS decreased the surface tension in fog water samples by 20% at 100 mg C L^{-1} (Facchini et al., 2000).

Baduel et al. (2012) measured the surface tensions in summer samples, which would be consistent with the high biogenic activity observed during this season. Ahern et al. (2007) showed that fluorescent pseudomonads isolated from clouds and rainwater produce biosurfactants. This study was the first and sole report on the exploration of the potential production of biosurfactants by microorganisms isolated from the cloud environment. Biosurfactants could be both directly issued from the Earth's surface during aerosolization or directly produced in cloud waters. However, a multitude of bacteria, fungi and yeasts display metabolic activities in clouds (Amato et al., 2005, 2007a; Hill et al., 2007; Sattler et al., 2001; Vaïtilingom et al., 2012, 2013). These microorganisms survive and resist atmospheric stresses (Delort et al., 2010; Joly et al., 2015).

Poorly considered in the atmosphere, biosurfactants have been extensively studied in soil- and plant-associated environments. Indeed, biosurfactant-producing bacteria in both undisturbed and contaminated soils have been well characterized (Bodour et al., 2003; Raaijmakers et al., 2010). Biosurfactants have been investigated for their capacity to remove heavy metals, and bioremediation is one of the main industrial applications of biosurfactants (Banat et al., 2010; Mulligan, 2009). Moreover, in terms of microbial life and activity, biosurfactants play a key role in bacterial cell motility, organic compound solubilization, microbial biofilm formation and disruption or anti-microbial activity (Chrzanowski et al., 2012; D'aes et al., 2010; Mann and Wozniak, 2012; Raaijmakers et al., 2010; Ron and Rosenberg, 2001).

Within a project aimed at examining atmospheric surfactants and characterizing their effects on cloud droplet formation, we focused on the biosurfactant-producing microorganisms present in atmospheric waters. Cloud

water samples are collected at the puy de Dôme station (France) belonging to the GAW (Global Atmosphere Watch) stations network. A total of 480 bacterial and yeast strains were isolated and identified. This unique collection of microorganisms was screened to identify biosurfactant-producing microorganisms. The surface tension of crude extracts, comprising supernatants of the pure culture, was determined using the pendant drop technique (Hansen and Rødsrud, 1991). We observed a potential correlation between the composition of cloud waters and the presence of biosurfactant-producing microorganisms. Finally, we discuss the potential impact of the production of biosurfactants by cloud microorganisms on atmospheric physicochemical processes.

2 Materials and Methods

2.1 Cloud sampling and physicochemical characterization of the cloud water samples

Cloud water samples were collected using a cloud droplet impactor sterilized by autoclaving and installed on the summit of the puy de Dôme Mountain (1465 m above sea level, 45°46'19"N, 2°57'52"E, Massif Central). Non-precipitating and non-convective cloud samples were collected. The experiments were conducted using the 480 microbial strains collected during 39 cloud events from 2004 to 2014. The physicochemical content of the aqueous cloud samples was characterized (concentrations of organic acids, inorganic ions and pH, see Table S1 in the supplement). Details about the sampling site, instrumentation and procedures for cloud sampling as well as the methods for the chemical analysis of cloud water samples, are provided in Deguillaume et al. (2014).

2.2 Isolation and identification of microorganisms from cloud waters

Triplicate volumes of 0.1 mL of cloud water were plated onto R2A agar growth medium (Reasoner and Geldreich, 1985; DIFCO™), and eventually onto R2A medium supplemented with NaCl 20 g L^{-1} and King's B (King et al., 1954), Sabouraud (DIFCO™) and TSA (Trycase Soy Agar, DIFCO™) 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.

Representative colonies were selected based on colony morphology and pigment production. The isolates obtained in pure cultures (R2A, 17°C) were stored in 10% (v/v) glycerol at -80°C. The strains were identified by ribosomal RNA gene sequencing (16S or 26S rRNA gene sequences for bacteria and yeasts, respectively). A complete description of the methods of identification is available in Vaïtilingom et al. (2012).

2.3 Surface tension measurements

Strains from the glycerol stocks were used to inoculate R2A broth in 96 deep-well plates (500 µL/well). The plates were incubated at 17°C under agitation for 5 days, followed by centrifugation (3000 g/20 min). The supernatants were transferred into 1-mL microtubes and stored at -30°C until subsequent surface tension measurements. The thawed samples were centrifuged (10,480 g/3 min) just prior to surface tension measurements.

All surface tension measurements were performed using the pendant drop method with an OCA 15 Pro tensiometer (Data Physics, Germany). The camera analyzes the pendant drop profile of the crude extract. A dosing needle with a 1.65-mm outside diameter was used, producing drops of 12 µL. The software fits this latter measurement to the Young-Laplace equation and averages out surface tension from all measurements (Hansen and Rødsrud, 1991). The measurements were obtained at 295 K every second. The tensiometer was calibrated using Milli-Q water. The uncertainty of the instrument was ± 0.01 mN m^{-1}. Each dynamic surface tension curve was measured three times for the most efficient biosurfactant-producing microorganisms, and the measurements displayed ± 10% variation. These dynamic surface tension measurements lasted until the equilibrium region is reached (maximum 30 min, see below section 3.2). Along with the surface tension, each measurement also provided real-time monitoring of the droplet volume, facilitating an assessment of the evaporation. No significant evaporation (< 5%) was observed during the experiments (Fig. 2).

3 Results

3.1 Identification of cloud microbial isolates

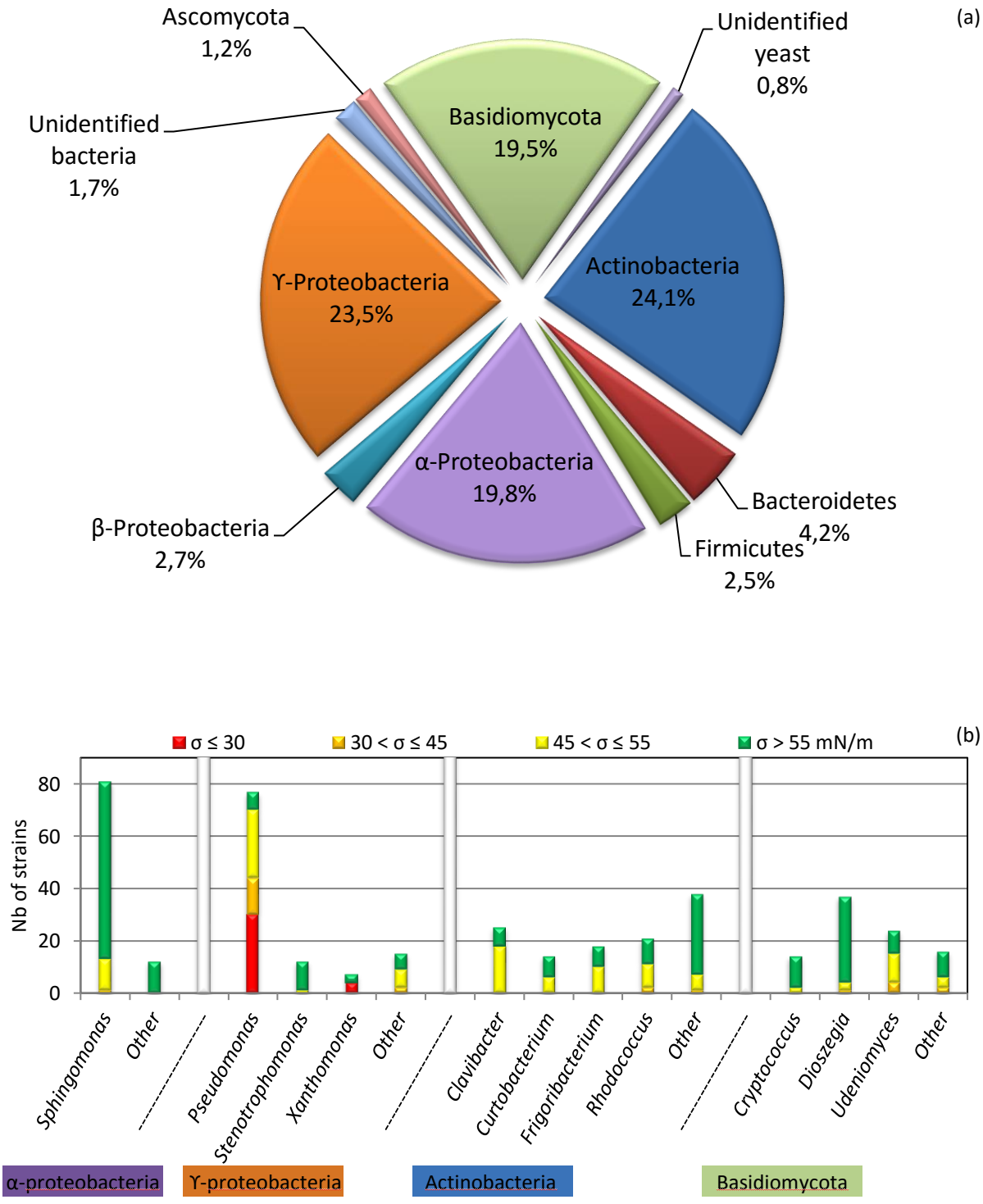

Figure 1. (a) Phyla distribution of the 480 strains examined for biosurfactant production. **(b)** Genera distribution of the most representative strains (85%: α- and Υ-Proteobacteria, Actinobacteria and Basidiomycota). The 4 categories of surface tensions ($\sigma \leq 30$, $30 < \sigma \leq 45$, $45 < \sigma \leq 55$ and $\sigma > 55$ mN m^{-1}) are indicated in red, orange, yellow and green, respectively.

The identification of the 480 strains (bacteria and yeasts) collected during the 39 cloud events at the puy de Dôme station, together with the values of surface tension obtained from their crude supernatants after 5 days of culture in R2A broth, is described in the Supplemental materials (Table S2).

Figure 1(a) shows the distribution of the different phyla or classes of these microbial isolates. Three phyla of microorganisms were dominant: Proteobacteria (α, β and ϒ- Proteobacteria), Actinobacteria and Basidiomycota, accounting for 89.6% of the collection, while 2.5% of the latter remain unidentified.

In detail (Fig. 1b), the phylum of Proteobacteria was predominant (220 isolates, 45.8%), particularly α- and ϒ-Proteobacteria (95 and 112 isolates, 19.8% and 23.3%, respectively). In these latter two classes, the most recurrent strains belonged to the genera *Sphingomonas* (83 isolates) and *Pseudomonas* (78 isolates), respectively. β-Proteobacteria represent only 2.7% of the total. The phylum Actinobacteria (116 isolates, 24.2%) primarily contained strains from the genera *Clavibacter* (25 isolates), *Curtobacterium* (14 isolates), *Frigoribacterium* (18 isolates) and *Rhodococcus* (21 isolates). Notably, the phylum Actinobacteria presents a much greater diversity of genera compared with the other phyla, centered on one dominant genus. Among the bacterial strains, the phyla Bacteroidetes and Firmicutes were also represented, but to a lesser extent (20 and 12 isolates, respectively, 4.2% and 2.5%).

Concerning yeasts, the major group belonged to the phylum Basidiomycota (94 isolates, 19.6%), primarily containing strains from the genera *Cryptococcus* (14 isolates), *Dioszegia* (39 isolates) and *Udeniomyces* (25 isolates). The phylum Ascomycota was also present but with only 6 isolates (1.2%).

Globally, the phylogeny of the isolated strains examined in the present study is consistent with the previously published phylogeny of the strains isolated from the same sampling sites, except for the genus *Bacillus*, which was much less abundant in the selected clouds events (Vaïtilingom et al., 2012). Notably, many strains originated from the phyllosphere, consistent with the predominance of the phylum Proteobacteria. These 480 strains therefore constitute a unique collection of cloud microorganisms, representative of a cloud community that can be tested for biosurfactant production.

3.2 **Screening for biosurfactant-producing microorganisms**

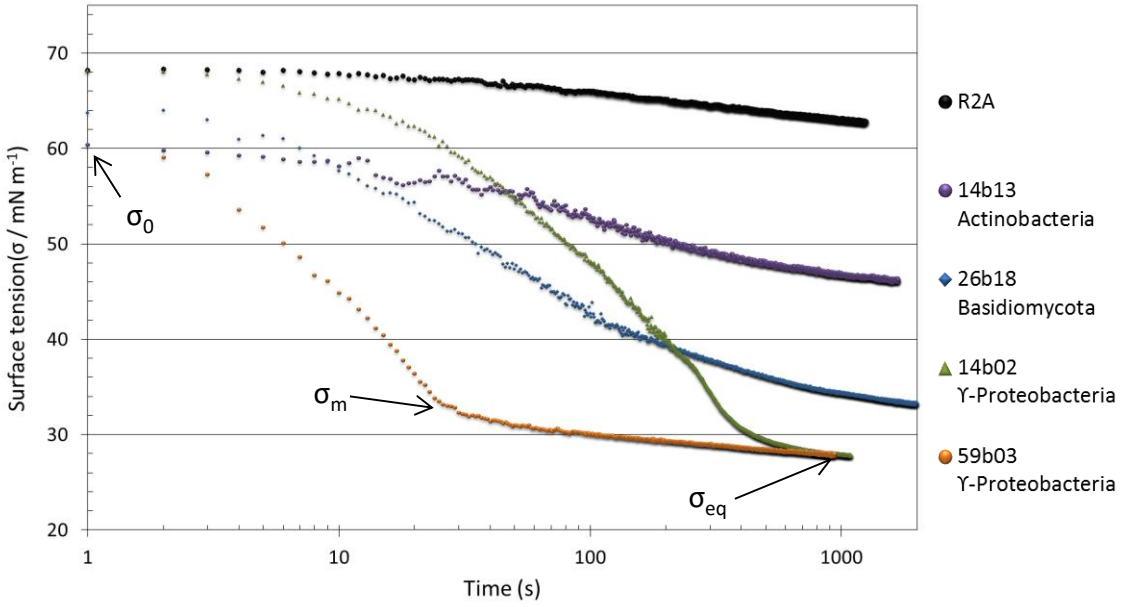

Figure 2. Time profile of the surface tension measurements. In black, R2A broth medium. In purple, strain 14b13 (*Frigoribacterium* sp., Actinobacteria). In blue, strain 26b18 (*Cryptococcus* sp., Basidiomycota). In green, strain 14b02 (*Pseudomonas* sp., ϒ-Proteobacteria). In orange, strain 59b03 (*Pseudomonas syringae*, ϒ-Proteobacteria). σ_0: initial surface tension. Surface tensions in the meso-equilibrium (σ_m) and equilibrium (σ_{eq}) phases.

Figure 2 shows the time profile of the surface tension measurements performed on the culture supernatants of 4 selected microbial strains. As a reference, the surface tension obtained from R2A medium, which served as the culture medium, is also presented. In this case, the observed surface tension (63 mN m^{-1}) remained close to the value of pure water (72.8 mN m^{-1}). As expected, a more or less strong reduction of the surface tension values was observed when the microorganisms produced biosurfactants in the culture medium. This phenomenon is time-dependent, and the time profiles were dependent on the studied strain. These profiles were consistent with those obtained from atmospheric aerosol by Nozière et al. (2014) and are typical of a surface tension dynamic (Hua and Rosen, 1991). Indeed, three distinct kinetic regimes follow each other during the equilibration process: first, a rapid decline of the R2A value to σ_0 occurs, which happens too rapidly to record (< 0.1 s). This is followed by the meso-equilibrium phase, during which the surface tension decreases to σ_m. Then, the appearance of the equilibrium region occurs, where the minimum, σ_{eq}, is reached. This region corresponds to the saturation of the surface (Γ_∞) with surfactant molecules. Hereafter, the surface tension measurements (σ) are referred to as σ_{eq}.

Monitoring the surface tension over time revealed that the equilibration time (apparent diffusion coefficient) in the pendant drops varied from a few seconds to 30 min, likely depending on the concentration and chemical structure of the expressed biosurfactants, which affect molecular interactions and/or diffusion. For example, from the supernatant of the strains 59b03 and 14b02, two *Pseudomonas* strains (ϒ-Proteobacteria), the measured

equilibrium surface tensions (σ_{eq}) were close (below 28 mN m^{-1}), while the time profiles were much different, and the equilibration stage occurred at approximately 2 and 10 minutes, respectively.

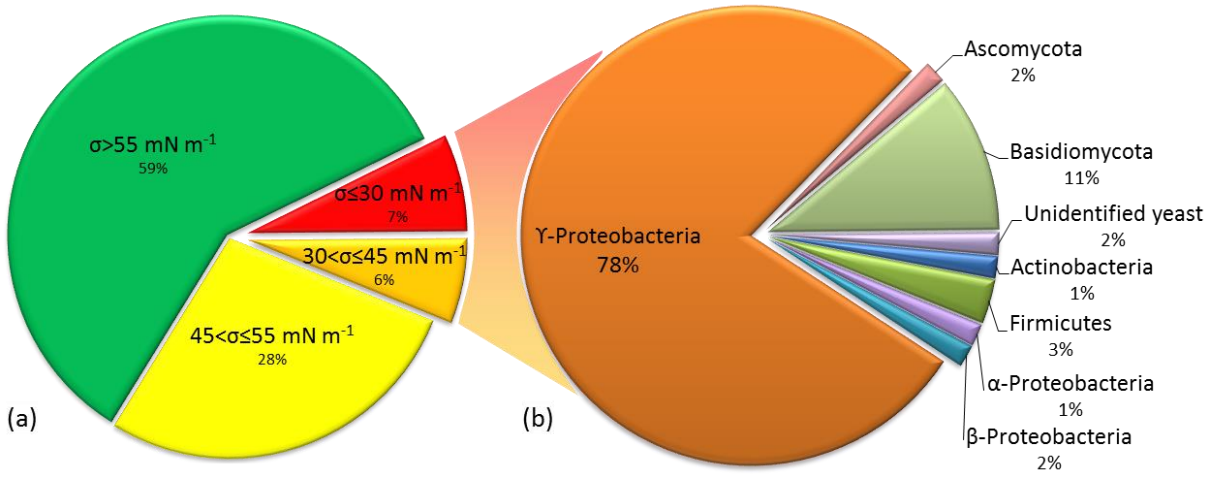

Figure 3. (a) Surface tension (σ) distribution of the 480 strains examined for biosurfactant production and **(b)** the phylum distribution for the most efficient biosurfactant-producing microorganisms ($\sigma \leq 45$ mN m^{-1}).

The 480 strains tested for biosurfactant production (see Supplement Table S2) were differentiated into four main categories according to the measured surface tension ($\sigma \leq 30$, $30 < \sigma \leq 45$, $45 < \sigma \leq 55$ and $\sigma > 55$ mN m^{-1}, Fig. 1b and Fig. 3). 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* (ϒ-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 ϒ-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 $\sigma > 55$ 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). Remarkably, *Pseudomonas* (ϒ-Proteobacteria) and *Sphingomonas* (α-Proteobacteria), the most frequently observed genera in the clouds (Vaïtilingom et al., 2012), showed completely different behaviors: *Pseudomonas* provide the most active biosurfactant-producing microorganisms, while almost all *Sphingomonas* are not efficient for the production of biosurfactants.

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).

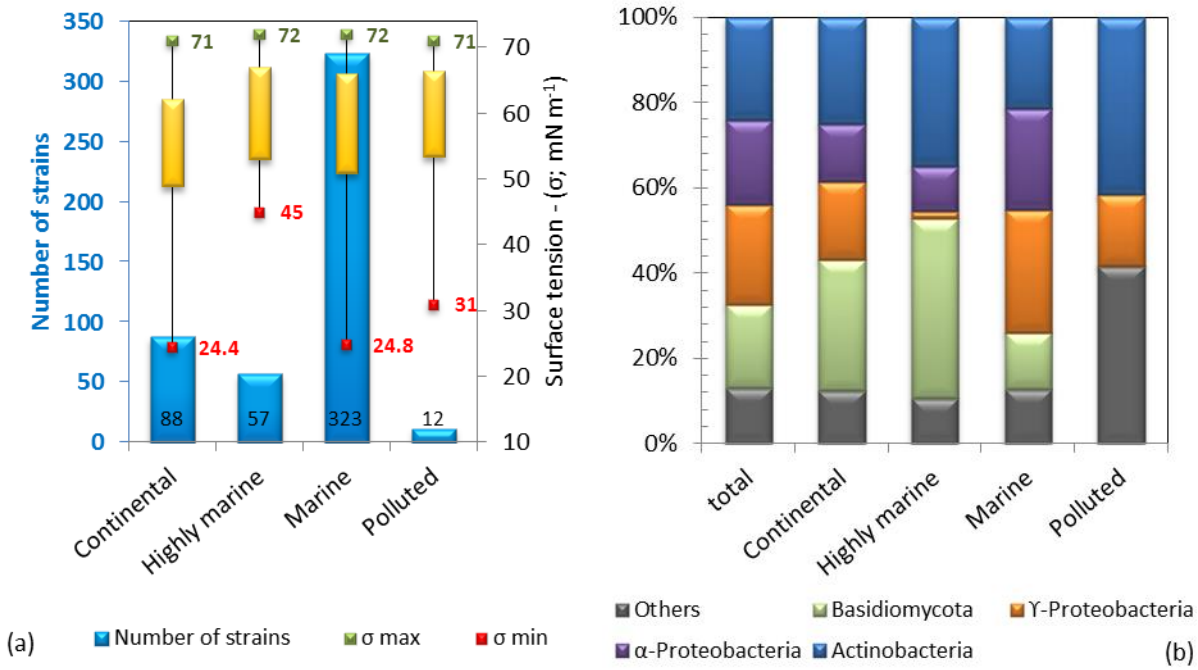

Figure 4 (a). Surface tension (σ) distribution of the 480 strains examined for biosurfactant production according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted). Highlighted in blue, the number of tested strains. Box and whisker plots are shown 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 ϒ-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.

4 Discussion and conclusion

The study of biosurfactant production in the environment has primarily focused on microorganisms isolated from soils, rhizospheres and the phyllosphere or from the marine environment (Bodour et al., 2003; Jackson et al., 2015; Raaijmakers et al., 2010; Satpute et al., 2010). Concerning atmospheric waters, Ahern et al. (2007) reported the presence of biosurfactant-producing bacteria in the air, studying 100 strains isolated from 4 rain and cloud samples. Here, we investigated 480 strains isolated from 39 different cloud events. When we consider that these strains typically produce biosurfactants when the measured surface tension (σ_{eq}) is less than 55 mN m^{-1}, 41% of the tested strains were active, 7% being extremely active ($\sigma < 30$ mN m^{-1}). Although the methods used to evaluate biosurfactant production were different, this result is consistent with that of Ahern et al. (2007), who reported 55% active strains in rain and cloud samples. In the present study, we showed that under laboratory conditions, the most efficient biosurfactant-producing microorganisms ($\sigma < 45$ mN m^{-1}) belonged to a limited number of bacterial genera (*Pseudomonas* and *Xanthomonas)* from the ϒ-Proteobacteria class (78%) and a yeast genus (*Udeniomyces*) from the Basidiomycota phylum (11%). Some *Bacillus* strains from the Firmicutes phylum were also active but represented a small fraction (3%) of the total population of the cloud collection examined in the present study. Strains from the Actinobacteria phylum were primarily present in the group with moderate biosurfactant production ($45 < \sigma < 55$ mN m^{-1}). In previous studies, *Pseudomonas* (ϒ-Proteobacteria) and *Bacillus* (Firmicutes) have been reported as high biosurfactant-producing microorganisms; *Acinetobacter* (α-Proteobacteria) has also been frequently reported (Desai and Banat, 1997; Rosenberg and Ron, 1999). For yeasts, the major high biosurfactant-producing genera include *Candida* and *Torulopsis* from phylum Ascomycota (Desai and Banat, 1997; Karanth et al., 1999; Rosenberg and Ron, 1999). In the present study, *Pseudomonas* strains were clearly the dominant group and the most active biosurfactant-producing microorganisms, whereas the *Acinetobacter* genus was absent. This result is highly consistent with studies performed on environmental samples, such as soils (Bodour et al., 2003), plants (D'aes et al., 2010; Raaijmakers et al., 2010), seawater (Cai et al., 2015) and in atmospheric water (Ahern et al., 2007).

Notably, the *Pseudomonas* genus is commonly detected in the phyllosphere, the main source of primary bioaerosols (Amato et al., 2007b; Morris et al., 2014; Vaïtilingom et al., 2012). Interestingly, biosurfactants play a specific role in the interactions between plants and *Pseudomonas* (D'aes et al., 2010; Raaijmakers et al., 2010). Biosurfactants present versatile functions, including interactions with other organisms (such as antibiotic activity) and modifications of the leaf-surface properties. These surface modifications enable cell mobility, biofilm formation and the colonization of the leaves by these bacteria. Particularly, rhamnolipids are involved in different processes of biofilm formation; the final step involves the release of the planktonic daughter population

(Mann and Wozniak, 2012). This production of biosurfactants could therefore be important for the formation of biofilms on leaf surfaces, facilitating the aerosolization and dispersion of *Pseudomonas* strains in the air.

This aerosolization of *Pseudomonas* strains could explain the correlation observed between the clouds composition and the distribution of biosurfactant-producing microorganisms observed in the present study. It is clear that microbial isolates from highly marine clouds, significantly impacted by the ocean source (an almost total absence of ϒ-Proteobacteria, see Fig. 4b), are lower biosurfactant producers than microorganisms isolated from continental clouds. Continental clouds, travelling over vegetated zones, thus contain more *Pseudomonas* strains. More generally, the correlation between the different origins of the air masses and the production of biosurfactants by cloud microorganisms could be explained by the significant differences in the vegetation of France. For example, the predominance of the most efficient biosurfactant-producing microorganisms in clouds originating from the Northwest-North sector could reflect agricultural practices. These French regions are characterized by uniform monocultures (Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt, 2014) extremely favorable to plant pathogens, such as *Pseudomonas syringae* (McDonald and Linde, 2002).

The occurrence of biosurfactants has recently been shown in atmospheric aerosols (Baduel et al., 2012; Ekström et al., 2010; Nozière et al., 2014). Indeed, the presence of biosurfactants in the atmosphere could result from different mechanisms. First, these aerosols could be directly produced by microorganisms in the clouds. This idea is relevant considering that microorganisms are metabolically active in clouds and can survive under atmospheric conditions (Joly et al., 2015 and references hereinafter). Indeed, microbial metabolic activity has been demonstrated by measurements of the ATP (adenosine 5'triphosphate) content (Amato et al., 2005, 2007a, Vaïtilingom et al., 2012, 2013) and by the uptake of the dye CTC (5-cyano-2,3-ditolyl tetrazolium chloride) directly *in situ* in cloud water samples (Hill et al., 2007). This activity has even been demonstrated at low temperatures (Sattler et al., 2001). In addition, these microorganisms also survive and resist atmospheric stresses, including evaporation-condensation cycles, freeze-thaw cycles, and exposure to oxidants and solar light and cold temperature (Delort et al., 2010; Joly et al., 2015). Biosurfactants can also be produced in extreme environments, such as deep sea or Arctic soil (Jackson et al., 2015; Janek et al., 2013). Under laboratory conditions, the microorganisms produced biosurfactants within the first 24 hours, even in poor nutritive medium (R2A). As the residence time of microorganisms in the atmosphere was modeled between 2 to 10 days (Burrows et al., 2009), these metabolically active microorganisms could thus synthesize biosurfactants in the clouds. A second and obvious pathway for the incorporation of biosurfactants in the atmosphere is associated with the presence of these molecules at the surface of the microorganisms. Biosurfactants can therefore be carried together with the microorganism when aerosolized or in a biofilm existing on dust or leaf particles. Moreover, biosurfactants could be directly emitted into the atmosphere as biogenic aerosols, particularly biosurfactants of marine origin, which can be emitted in sea sprays during bubbling and wave braking processes (Blanchard, 1989; Elliott et al., 2014).

The presence of biosurfactants might have implications for atmospheric processes. First, these molecules could impact atmospheric chemistry. For example, the *in situ* biosynthesis of biosurfactants by microorganisms in clouds could be considered as the production of secondary organic aerosols, thus modifying the global carbon balance in the water phase. The presence of biosurfactants (aerosolized from the earth surface or produced *in situ)* at the surface of cloud droplets could also change the precise picture of the mass transfer between the gas and water phases of clouds. Organic surface films can provide barriers against transportation across the air-

particle interface, inhibiting the uptake of water and gas phase species. These organic films can be an auspicious medium for solubilizing gas phase organic species, perhaps reflecting the observed non-Henry's law concentration of organics in field samples (Barnes, 1986; Davies et al., 2013; Lo and Lee, 1996; Park et al., 2009; Renard et al., 2014). The chemical characterization and the study of the reactivity of these organic layers will be of special interest to further understand cloud chemistry.

Second, biosurfactants could affect atmospheric microphysics by modifying CCN activation. Owing to their exceptional scope in reducing surface tension, biosurfactants *per se*, whether present on aerosols or associated at the microorganisms surface, are thus likely to enhance the propensity of these aerosols to form clouds, as the activation of particles into cloud droplets depends on surface tension according to Köhler's theory (Köhler, 1936). This topic has been controversial, but recently, Nozière et al. (2014) showed that the total surfactant fraction of atmospheric particles is much more surface-active ($\sigma \leq 30$ mN m^{-1}) than HULIS. These authors demonstrated that the equilibration time of biosurfactants might hinder the measurement of such an effect when using classical instruments, such as a hygroscopic tandem differential mobility analyzer or a CCN counter. Upon further examination of the results obtained in the present study, it is reasonable to consider that biosurfactant partitioning in macroscopic pendant droplets might decrease the surface tension values relative to atmospheric conditions. Indeed, in a microscopic ($D_{wet} \approx 1$ µm) droplet, the partitioning impact could be insignificant owing to a surface area to volume ratio several orders of magnitude higher (Prisle et al., 2012; Sorjamaa et al., 2004). Nevertheless, the lower the radius of the droplet or the higher the surface to volume ratio, the higher the bacteria concentration (Aller et al., 2005; Hejkal et al., 1980) and the higher the WSOC concentration (Ervens and Volkamer, 2010). By dividing the surfactant concentration in the atmosphere (~10^{-12} – 10^{-9} mol m^{-3} in Olkowska et al., 2014) by the liquid water content of wet aerosol (~10^{-6} – 10^{-5} g m^{-3} in Ervens and Volkamer, 2010), we obtained a significant concentration of ~mM. A recent study (Gérard et al., 2016) reported concentrations above the typical CMC. Thus, Ruehl et al. (2012) presented strong evidence that surface tension reduction can occur in microscopic droplets, and even more in wet aerosol, provided that the particles predominantly (*i.e.*, $\gtrsim$ 80%) comprise surfactants.

The influence of biological surfactants on the prediction of particle cloud activation and indirect aerosol climate effects should be implemented in models. Indeed, if the effects of organic surfactants (particularly carboxylic acids) on the surface tension of activating droplets is considered in parameterizations (Abdul-Razzak and Ghan, 2004), then recent studies have shown that the surface partitioning of organic molecules to a microscopic aqueous droplet interface should also be considered in models (Noziere, 2016; Prisle et al., 2012; Ruehl et al., 2016).

Moreover, because *Pseudomonas* strains are the most efficient biosurfactant-producing bacteria and are dominant in clouds and rain (the present study and Ahern et al., 2007), the question arises about the potential role of biosurfactants in the cycle of *Pseudomonas* in the atmosphere. Indeed, biosurfactants facilitate the aerosolization of theses strains from leaf surfaces and favor the formation of cloud droplets through the modification of the surface properties of the cells. Moreover, most of these strains belong to *Pseudomonas syringae* species, which are ice nuclei active and can induce precipitations back to the earth. Thus, biosurfactants

should be integrated into the life history of *Pseudomonas syringae* and its relationship to the water cycle (Morris et al., 2008, 2014).

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. These comparisons should provide evidence of the microbial origin of the surfactants present on aerosols. Studying such biosurfactants in the atmosphere is of special interest for the chemical characterization and reactivity of organic layers and the characterization of their impact on mass transfer and water uptake. The activation of aerosols containing organic matter is a major topic directly associated with the climatic effects of aerosol-cloud-interactions. A small change in the droplet population could affect cloud albedo and the formation of precipitation (Li et al., 2011; Rodhe, 1999). Hence, there is a need to enhance our knowledge about biosurfactants, focusing on the extent of their impact on human health and the global climate (Brimblecombe and Latif, 2004).

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Screening of cloud microorganisms isolated at the puy de Dôme (France) station for the production of biosurfactants

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Table S1. Strains are isolated during 39 cloud events (from 2004 to 2014) gathered in four categories according to the physicochemical characteristics of the cloud waters (Blue: marine, purple: highly marine, green: continental and black: polluted) as described by Deguillaume et al. (2014).

Cloud Event	Composition	Nb of strains	Date	pH	Ions (µM)												
					SO_4^{2-}	NO_3^-	Cl^-	Acetate	Formate	Oxalate	Succinate	Malonate	Na^+	NH_4^+	Mg^{2+}	K^+	Ca^{2+}
21	Marine	2	2004-01	5.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
23	Polluted	2	2004-02	3.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
29	Marine	1	2004-07	5.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	Marine	3	2004-09	7.6	3.8	6.5	12.0	6.0	6.0	0.5	0.1	0.16	19.4	54.9	4.9	4.1	12.0
32	Continental	1	2004-12	5.5	72.1	95.8	31.5	0.0	0.3	0.3	0.1	0.17	74.4	132.4	7.6	9.0	73.7
42	Continental	25	2007-12	4.7	39.7	198.4	20.2	10.2	5.8	2.9	0.6	0.58	19.1	148.2	3.5	11.9	58.0
43	Highly marine	25	2008-01	5.9	9.4	21.4	81.4	11.4	6.7	1.2	0.2	0.28	315.7	35.9	11.8	13.7	26.0
44	Continental	14	2008-02	5.2	24.6	65.9	17.2	26.8	18.0	1.3	0.5	0.39	15.2	148.5	1.8	5.8	22.3
45	Continental	22	2008-04	4.6	44.7	65.5	31.7	17.7	17.9	2.8	0.9	0.89	33.3	122.5	4.9	9.7	53.6
46	Continental	2	2008-10	5.0	13.2	102.4	28.7	2.7	14.2	1.3	0.4	0.52	10.4	72.7	6.9	5.3	77.7
47	Marine	14	2008-11	5.4	6.5	33.2	15.7	5.3	8.0	0.8	0.2	0.33	8.2	13.7	1.4	6.3	14.4
49	Marine	6	2009-01	6.5	14.7	20.0	113.9	4.8	8.9	1.2	0.3	0.44	70.9	58.2	29.2	12.5	14.5
50	Marine	7	2009-02	4.9	7.1	23.3	72.5	22.2	13.4	0.8	0.2	0.56	NA	NA	NA	NA	NA
53	Polluted	8	2009-03	4.0	73.8	516.5	193.8	41.2	13.7	3.5	1.2	0.62	171.9	363.2	13.5	71.6	52.3
54	Marine	62	2009-11	5.2	2.3	13.3	30.5	4.4	15.0	1.7	0.1	0.34	37.0	6.6	12.1	20.5	0.0
55	Marine	11	2009-11	5.8	9.3	34.8	97.5	6.7	10.1	3.3	0.3	0.36	95.1	31.1	12.6	17.2	0.0
60	Highly marine	32	2010-03	5.5	39.0	9.7	231.5	3.5	4.3	1.2	0.0	0.00	114.1	28.6	12.9	12.8	2.7
61	Marine	2	2010-05	6.2	3.1	6.0	11.6	6.0	6.4	0.1	0.0	0.00	11.8	15.4	0.2	6.2	4.2
62	Marine	1	2010-06	6.1	3.5	4.5	2.3	3.4	6.2	1.4	0.0	0.00	1.8	6.0	0.0	0.0	0.0
66	Marine	1	2010-09	5.7	4.0	17.8	1.5	7.1	8.6	2.6	0.2	0.00	2.3	32.4	0.2	0.7	0.5
71	Marine	2	2011-03	5.9	3.5	6.6	6.2	7.1	4.2	2.2	0.3	0.39	6.9	42.5	0.1	3.6	5.1
72	Marine	5	2011-03	7.0	12.7	26.0	26.4	16.6	12.7	1.8	0.5	0.53	40.2	75.1	0.4	12.5	15.2
75	Marine	9	2011-06	5.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
76	Continental	4	2011-06	5.9	52.2	126.0	16.9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
77	Continental	11	2011-07	6.0	19.6	47.9	12.8	58.3	109.6	12.3	NA	NA	124.8	408.5	26.8	22.8	148
78	Marine	8	2011-07	5.5	7.1	10.4	24.1	3.3	11.3	3.1	NA	NA	22.7	32.7	12.3	9.2	7.1
79	Marine	5	2011-11	4.6	1.3	5.0	0.3	4.3	5.6	1.0	NA	NA	0.6	52.7	0.8	1.6	3.2
80	Marine	2	2012-01	4.9	1.8	2.2	13.4	7.4	5.3	1.1	NA	NA	80.6	39.3	12.0	6.1	5.2
81	Marine	7	2012-01	5.8	4.8	11.2	23.4	15.3	15.6	6.7	NA	NA	145.7	188.6	20.3	6.2	9.3
82	Marine	2	2012-03	5.3	1.6	2.0	0.2	4.2	6.2	3.0	NA	NA	1.0	75.4	1.0	0.1	6.1
83	Continental	10	2012-04	5.6	10.7	49.9	12.2	0.0	39.0	6.5	NA	NA	93.5	531.1	14.3	10.7	34.6
84	Marine	21	2012-04	5.5	1.6	1.9	6.0	3.9	7.7	1.2	NA	NA	36.0	38.1	6.0	1.4	5.2
85	Marine	17	2012-06	5.5	1.2	3.0	0.9	0.0	18.2	3.3	NA	NA	6.0	77.9	2.2	4.0	4.3
86	Marine	42	2012-09	5.9	0.5	1.0	1.0	3.2	3.2	1.0	NA	NA	8.8	16.8	1.4	5.5	4.5
87	Marine	28	2012-10	6.2	5.3	11.0	26.7	4.0	13.6	0.7	NA	NA	21.2	39.3	8.3	7.6	2.3
88	Polluted	2	2012-11	4.6	111.1	346.5	47.6	18.5	13.1	6.5	NA	NA	17.6	59.3	15.8	53.6	6.6
89	Marine	31	2013-01	5.2	32.9	29.5	109.9	19.9	15.6	2.7	NA	NA	77.5	81.2	21.5	4.8	23.5
91	Marine	7	2013-05	5.5	28.5	9.3	16.3	21.8	9.8	3.3	NA	NA	13.3	57.4	6.2	8.0	22.2
97	Marine	26	2014-02	5.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S2. 480 strains tested for biosurfactant production. Strains are isolated during 39 cloud events (from 2004 to 2014) gathered in four categories according to the physicochemical characteristics of the cloud waters (Blue: Marine, purple: Highly marine, green: Continental and black: Polluted, see table S1) as described by Deguillaume et al. (2014). Strains are differentiated into four main categories, according to the measured surface tension (red: $\sigma \leq 30$, orange: $30 < \sigma \leq 45$, yellow: $45 < \sigma \leq 55$ and green: $\sigma > 55$ mN m^{-1}, see details in text). Phylum colors correspond to those of Figure 1. All surface tension measurements are performed using the pendant drop method with an OCA 15 Pro tensiometer (Data Physics, Germany). A.N, accession number in GenBank.

Cloud Events	Composition	Strain	Phylum (class)	Species	AN	σ (mN m^{-1})
76	Continental	49b04	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922066	24.4
77	Continental	50b03	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922069	25.6
77	Continental	50b08	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922074	25.6
77	Continental	50b04	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922070	25.9
77	Continental	50b02	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922068	26.1
45	Continental	26b18	Basidiomycota	*Udeniomyces* sp.	JF706566	33
44	Continental	25b01	Basidiomycota	*Bullera globispora*	HQ260318	36
77	Continental	50b07	ϒ-Proteobacteria	*Pseudomonas syringae*	KR922073	36
42	Continental	23b16	Actinobacteria	*Leifsonia* sp.	HQ256777	39
77	Continental	50b09	ϒ-Proteobacteria	*Pseudomonas syringae*	KR922075	39
44	Continental	25b04	Basidiomycota	*Udeniomyces* sp.	HQ256877	39
76	Continental	49b03	Unidentified yeast	*unidentified*		42
44	Continental	25b05	ϒ-Proteobacteria	*Pseudomonas* sp.	HQ256806	43
45	Continental	26b25	β-Proteobacteria	*Variovorax* sp.	HQ256810	44
44	Continental	25b07	ϒ-Proteobacteria	*Erwinia billingiae*	HQ256807	46
44	Continental	25b11	ϒ-Proteobacteria	*Erwinia billingiae*	HQ256802	46
44	Continental	25b13	ϒ-Proteobacteria	*Erwinia billingiae*	HQ256804	46
77	Continental	50b05	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922071	48
42	Continental	23b26	unidentified bacteria	*unidentified*		48
44	Continental	25b03	Actinobacteria	*Clavibacter michiganensis*	HQ256805	49
45	Continental	26b30	Actinobacteria	*Clavibacter michiganensis*	HQ256811	49
45	Continental	26b21	Actinobacteria	*Plantibacter* sp.	HQ260322	49
42	Continental	23b05	Actinobacteria	*Rhodococcus* sp.	HQ256785	49
42	Continental	23b27	Actinobacteria	*Rhodococcus* sp.	HQ256783	49
83	Continental	56b23	Basidiomycota	*Udeniomyces* sp.		49
42	Continental	23b28	unidentified bacteria	*unidentified*		49
45	Continental	26b19	Basidiomycota	*Bullera globispora*	JF706567	50
45	Continental	26b16	α-Proteobacteria	*Sphingomonas* sp.	HQ256808	50
46	Continental	27b03	ϒ-Proteobacteria	*Pseudomonas* sp.	HQ256813	51
77	Continental	50b10	ϒ-Proteobacteria	*Pseudomonas* sp.		51
42	Continental	23b25	Actinobacteria	*Rhodococcus* sp.	HQ256782	51
83	Continental	56b01	Actinobacteria	*Plantibacter* sp.		52
83	Continental	56b13	Actinobacteria	*Rhodococcus* sp.		52
44	Continental	25b09	Basidiomycota	*Udeniomyces* sp.	HQ256880	52
83	Continental	56b08	Actinobacteria	*Clavibacter michiganensis*	KR922100	53
83	Continental	56b03	Actinobacteria	*Rhodococcus* sp.	KR922098	53
44	Continental	25b14	Basidiomycota	*Dioszegia fristingensis*		54
42	Continental	23b15	Actinobacteria	*Microbacterium oxydans*	HQ256776	54
42	Continental	23b20	Actinobacteria	*Microbacterium* sp.	HQ256779	54
45	Continental	26b08	Basidiomycota	*Cryptococcus* sp.	JF706563	55
77	Continental	50b06	ϒ-Proteobacteria	*Pseudomonas syringae*	KR922072	55
42	Continental	23b22	Basidiomycota	*Cryptococcus victoriae*	JF706548	56
44	Continental	25b06	Basidiomycota	*Dioszegia butyracea*	HQ256878	56
83	Continental	56b25	Actinobacteria	*Frigoribacterium* sp.	KR922104	56
45	Continental	26b32	Ascomycota	*Tetracladium* sp.	JF706575	56
45	Continental	26b04	Basidiomycota	*Dioszegia* sp.	JF706560	57
42	Continental	23b29	Actinobacteria	*Rhodococcus* sp.	HQ256784	57
77	Continental	50b11	α-Proteobacteria	*Sphingomonas* sp.		57
83	Continental	56b21	α-Proteobacteria	*Sphingomonas* sp.	KR922103	57
45	Continental	26b23	Actinobacteria	*Subtercola* sp.	HQ256809	57
45	Continental	26b11	Basidiomycota	*Cryptococcus* sp.	JF706565	58
42	Continental	23b18	Basidiomycota	*Cryptococcus victoriae*	JF706547	58
83	Continental	56b14	Actinobacteria	*Plantibacter* sp.	KR922102	58
83	Continental	56b04	α-Proteobacteria	*Sphingomonas* sp.	KR922099	58
42	Continental	23b21	Actinobacteria	*Subtercola boreus*	HQ256780	58
45	Continental	26b06	Basidiomycota	*Dioszegia* sp.	JF706562	59
45	Continental	26b34	Basidiomycota	*Dioszegia xingshenensis*	JF706577	59
44	Continental	25b12	Actinobacteria	*Rhodococcus* sp.	HQ256803	59
44	Continental	25b10	Basidiomycota	*Dioszegia* sp.	HQ256875	60

42	Continental	23b24	α-Proteobacteria	*Rhizobium* sp.	HQ256781	60
45	Continental	26b03	Basidiomycota	*Cryptococcus* sp.	JF706559	61
42	Continental	23b07	α-Proteobacteria	*Devosia* sp.	HQ256787	61
45	Continental	26b09	Basidiomycota	*Dioszegia buhagiarii*	JF706564	61
45	Continental	26b20	Basidiomycota	*Dioszegia* sp.	JF706568	62
45	Continental	26b24	Basidiomycota	*Dioszegia* sp.	JF706570	62
45	Continental	26b26	Basidiomycota	*Dioszegia* sp.	JF706571	62
45	Continental	26b05	Basidiomycota	*Cryptococcus* sp.	JF706561	63
42	Continental	23b13	Basidiomycota	*Dioszegia* sp.	JF706546	63
42	Continental	23b03	Ascomycota	*Unidentified*	JF706545	63
44	Continental	25b02	Basidiomycota	*Udeniomyces* sp.	HQ256876	64
44	Continental	25b08	Basidiomycota	*Dioszegia butyracea*	HQ256879	65
77	Continental	50b01	ϒ-Proteobacteria	*Pseudomonas grimondii*	KR922067	65
46	Continental	27b01	α-Proteobacteria	*Sphingomonas* sp.	HQ256812	65
76	Continental	49b02	α-Proteobacteria	*Sphingomonas* sp.	KR922065	65
76	Continental	49b01	Unidentified yeast	*unidentified*		65
42	Continental	23b09	Actinobacteria	*Streptomyces* sp.	HQ256788	66
42	Continental	23b14	α-Proteobacteria	*Methylobacterium* sp.	HQ256775	67
42	Continental	23b19	α-Proteobacteria	*Sphingomonas* sp.		67
83	Continental	56b24	unidentified bacteria	*unidentified*		67
42	Continental	23b12	Actinobacteria	*Streptomyces* sp.		68
42	Continental	23b02	Ascomycota	*Unidentified*	JF706544	68
42	Continental	23b06	α-Proteobacteria	*Sphingomonas* sp.	HQ256786	69
42	Continental	23b17	Bacteroidetes	*Hymenobacter* sp.	HQ256778	70
45	Continental	26b27	Basidiomycota	*Rhodotorula aurantiaca*	JF706572	70
42	Continental	23b11	Actinobacteria	*Streptomyces* sp.	HQ256774	70
45	Continental	26b31	Ascomycota	*Taphrina deformans*	JF706574	70
45	Continental	26b33	Basidiomycota	*Dioszegia* sp.	JF706576	71
42	Continental	23b01	α-Proteobacteria	*Sphingomonas* sp.	HQ256773	71
43	Highly marine	24b16	Ascomycota	*Wickerhamomyces anomalus*	JF706554	45
43	Highly marine	24b04	Actinobacteria	*Streptomyces microflavus*	HQ256797	47
43	Highly marine	24b12	ϒ-Proteobacteria	*Pseudomonas* sp.	HQ260323	49
43	Highly marine	24b19	Actinobacteria	*Clavibacter michiganensis*	HQ260320	50
43	Highly marine	24b26	α-Proteobacteria	*Sphingomonas* sp.		50
43	Highly marine	24b10	Actinobacteria	*Streptomyces microflavus*	HQ256790	50
43	Highly marine	24b13	Ascomycota	*Wickerhamomyces anomalus*	JF706551	50
43	Highly marine	24b17	Actinobacteria	*Clavibacter michiganensis*	HQ256792	51
43	Highly marine	24b24	Actinobacteria	*Clavibacter michiganensis*	HQ256795	51
43	Highly marine	24b20	Actinobacteria	*Frigoribacterium* sp.	HQ256793	51
43	Highly marine	24b18	Basidiomycota	*Udeniomyces pannonicus*	JF706555	51
43	Highly marine	24b21	Basidiomycota	*Udeniomyces pannonicus*	JF706556	51
43	Highly marine	24b05	Actinobacteria	*Clavibacter michiganensis*	HQ256798	52
43	Highly marine	24b15	Basidiomycota	*Dioszegia fristigensis*	JF706553	52
43	Highly marine	24b07	Actinobacteria	*Curtobacterium flaccumfaciens*	HQ256800	53
43	Highly marine	24b23	Actinobacteria	*Curtobacterium flaccumfaciens*	HQ256794	53
60	Highly marine	35b43	Actinobacteria	*Rhodococcus* sp.	JF706519	53
60	Highly marine	35b14	Firmicutes	*Bacillus* sp.		55
43	Highly marine	24b06	Actinobacteria	*Curtobacterium flaccumfaciens*	HQ256799	55
43	Highly marine	24b09	Actinobacteria	*Aeromicrobium* sp.	HQ256801	57
43	Highly marine	24b01	Actinobacteria	*Clavibacter michiganensis*	HQ256789	58
60	Highly marine	35b15	α-Proteobacteria	*Sphingomonas* sp.	JF706508	58
60	Highly marine	35b22	unidentified bacteria	*unidentified*		58
60	Highly marine	35b13	Actinobacteria	*Frigoribacterium* sp.	JF706507	59
43	Highly marine	24b08	Basidiomycota	*Bullera armeniaca*	JF706550	60
60	Highly marine	35b18	Actinobacteria	*Curtobacterium herbarum*	JF706509	60
60	Highly marine	35b40	β-Proteobacteria	*Janthinobacterium* sp.	JF706518	60
43	Highly marine	24b02	Basidiomycota	*Bullera armeniaca*	JF706549	61
60	Highly marine	35b26	Basidiomycota	*Udeniomyces* sp.	JN176601	61
43	Highly marine	24b22	Basidiomycota	*Bullera armeniaca*	JF706557	62
60	Highly marine	35b45	Basidiomycota	*Dioszegia fristigensis*	JN176610	63
43	Highly marine	24b25	Basidiomycota	*Udeniomyces* sp.	JF706558	63
60	Highly marine	35b29	Basidiomycota	*Dioszegia butyracea*	JN176603	64
60	Highly marine	35b35	Basidiomycota	*Dioszegia crocea*	JN176606	64
60	Highly marine	35b30	Basidiomycota	*Dioszegia crocea*	JN176604	65
60	Highly marine	35b02	α-Proteobacteria	*Sphingomonas* sp.	JF706510	65
60	Highly marine	35b39	Basidiomycota	*Dioszegia crocea*	JN176607	66
60	Highly marine	35b42	Basidiomycota	*Dioszegia fristingensis*	JN176608	66
60	Highly marine	35b01	Actinobacteria	*Frigoribacterium* sp.	JF706506	66
60	Highly marine	35b17	Basidiomycota	*Dioszegia crocea*	JN176597	67
60	Highly marine	35b44	Basidiomycota	*Dioszegia crocea*	JN176609	67
43	Highly marine	24b14	Basidiomycota	*Dioszegia* sp.	JF706552	67

60	Highly marine	35b21	Basidiomycota	*Mastigobasidium intermedium*	JN176599	67
60	Highly marine	35b23	Basidiomycota	*Mastigobasidium intermedium*	JN176600	67
60	Highly marine	35b27	α-Proteobacteria	*Methylobacterium* sp.	JF706512	67
60	Highly marine	35b20	Actinobacteria	*Curtobacterium flaccumfaciens*	JF706511	68
60	Highly marine	35b33	β-Proteobacteria	*Massilia* sp.	JF706514	68
60	Highly marine	35b38	α-Proteobacteria	*Sphingomonas* sp.	JF706517	68
60	Highly marine	35b32	α-Proteobacteria	*Sphingomonas* sp.	JF706513	69
60	Highly marine	35b12	Basidiomycota	*Sporobolomyces roseus*	JF706594	69
43	Highly marine	24b03	Actinobacteria	*Streptomyces* sp.	HQ256796	69
60	Highly marine	35b04	Basidiomycota	*Cryptococcus* sp.	JN176596	70
60	Highly marine	35b19	Basidiomycota	*Dioszegia crocea*	JN176598	70
60	Highly marine	35b28	Basidiomycota	*Dioszegia fristigensis*	JN176602	70
60	Highly marine	35b34	Actinobacteria	*Rhodococcus* sp.	JF706515	71
60	Highly marine	35b37	Actinobacteria	*Rhodococcus* sp.	JF706516	71
60	Highly marine	35b31	Basidiomycota	*Dioszegia crocea*	JN176605	72
78	Marine	51b07	Υ-Proteobacteria	*Pseudomonas* sp.	KR922082	24.8
78	Marine	51b04	Υ-Proteobacteria	*Pseudomonas* sp.	KR922079	25.2
87	Marine	60b24	Υ-Proteobacteria	*Pseudomonas* sp.	KR922197	25.3
54	Marine	32b42	Υ-Proteobacteria	*Pseudomonas* sp.	HQ256842	25.5
75	Marine	48b01	Υ-Proteobacteria	*Pseudomonas* sp.	KR922057	25.7
78	Marine	51b06	Υ-Proteobacteria	*Pseudomonas* sp.	KR922081	26
87	Marine	60b01	Υ-Proteobacteria	*Pseudomonas* sp.	KR922180	26
85	Marine	58b28	Υ-Proteobacteria	*Pseudomonas syringae*	KR922139	26
85	Marine	58b02	Υ-Proteobacteria	*Pseudomonas syringae*	KR922125	26.4
86	Marine	59b12	Υ-Proteobacteria	*Pseudomonas* sp.	KR922150	26.8
86	Marine	59b10	Υ-Proteobacteria	*Pseudomonas* sp.	KR922148	27
54	Marine	32b53	Υ-Proteobacteria	*Pseudomonas trivialis*	HQ256851	27
54	Marine	32b52	Υ-Proteobacteria	*Xanthomonas campestris*	HQ256850	27
61	Marine	36b03	Υ-Proteobacteria	*Pseudomonas fluorescens*	JF706525	27.5
86	Marine	59b11	Υ-Proteobacteria	*Pseudomonas* sp.	KR922149	27.5
91	Marine	66b02	Υ-Proteobacteria	*Pseudomonas syringae*	KR922247	27.5
75	Marine	48b02	Υ-Proteobacteria	*Pseudomonas reinekei*		27.8
30	Marine	14b02	Υ-Proteobacteria	*Pseudomonas* sp.		27.8
72	Marine	47b07	Υ-Proteobacteria	*Pseudomonas* sp.		27.8
78	Marine	51b03	Υ-Proteobacteria	*Pseudomonas* sp.	KR922078	28
54	Marine	32b32	Υ-Proteobacteria	*Xanthomonas campestris*	JN176586	28.3
86	Marine	59b03	Υ-Proteobacteria	*Pseudomonas syringae*	KR922141	28.4
54	Marine	32b22	Υ-Proteobacteria	*Xanthomonas campestris*	JN176582	29
61	Marine	36b05	Υ-Proteobacteria	*Pseudomonas fluorescens*	JF706526	29.1
54	Marine	32b74	Υ-Proteobacteria	*Pseudomonas syringae*	HQ256872	29.1
84	Marine	57b01	Υ-Proteobacteria	*Pseudomonas* sp.	KR922105	30
86	Marine	59b37	Υ-Proteobacteria	*Pseudomonas* sp.	KR922166	30
55	Marine	33b02	Υ-Proteobacteria	*Pseudomonas syringae*	HQ256867	30
54	Marine	32b24	Υ-Proteobacteria	*Xanthomonas* sp.	JN176583	30
91	Marine	66b05	Υ-Proteobacteria	*Pseudomonas graminis*	KR922249	33
78	Marine	51b05	Υ-Proteobacteria	*Pseudomonas syringae*	KR922080	33
82	Marine	55b15	Υ-Proteobacteria	*Pseudomonas syringae*	KR922097	34
86	Marine	59b32	Υ-Proteobacteria	*Erwinia billingiae*	KR922162	35
75	Marine	48b05	Υ-Proteobacteria	*Pseudomonas syringae*	KR922059	35
86	Marine	59b16	Υ-Proteobacteria	*Pseudomonas syringae*	KR922153	36
54	Marine	32b27	Basidiomycota	*Dioszegia xingshenensis*	JN176593	39
21	Marine	05b01	Firmicutes	*Bacillus pumilus*		40
86	Marine	59b07	Υ-Proteobacteria	*Pseudomonas* sp.	KR922145	40
86	Marine	59b25	Υ-Proteobacteria	*Pseudomonas graminis*	KR922157	41
78	Marine	51b01	Υ-Proteobacteria	*Pseudomonas* sp.	KR922077	41
54	Marine	32b67	Υ-Proteobacteria	*Pseudomonas syringae*	HQ256864	42
47	Marine	28b11	Basidiomycota	*Udeniomyces pannonicus*	HQ256882	42
86	Marine	59b41	Υ-Proteobacteria	*Erwinia* sp.	KR922170	43
47	Marine	28b12	Basidiomycota	*Udeniomyces* sp.	HQ256883	43
86	Marine	59b14	Υ-Proteobacteria	*Pseudomonas syringae*	KR922151	44
47	Marine	28b02	Basidiomycota	*Bannoa* sp.	JN176592	45
85	Marine	58b01	Υ-Proteobacteria	*Pseudomonas syringae*	KR922124	45
91	Marine	66b14	α-Proteobacteria	*Sphingomonas* sp.	KR922251	45
84	Marine	57b26	Actinobacteria	*Clavibacter michiganensis*	KR922121	46
30	Marine	14b13	Actinobacteria	*Frigoribacterium* sp.	DQ512796	46
86	Marine	59b02	Υ-Proteobacteria	*Pseudomonas syringae*		46
85	Marine	58b25	Υ-Proteobacteria	*Pseudomonas* sp.	KR922137	47
86	Marine	59b04	Υ-Proteobacteria	*Pseudomonas* sp.	KR922142	47
78	Marine	51b10	Υ-Proteobacteria	*Pseudomonas syringae*	KR922084	47
86	Marine	59b05	Υ-Proteobacteria	*Pseudomonas syringae*	KR922143	47
49	Marine	29b06	Basidiomycota	*Udeniomyces pannonicus*	HQ256895	47

54	Marine	32b64	Basidiomycota	*Udeniomyces* sp.	JF706586	47
84	Marine	57b22	Actinobacteria	*Clavibacter michiganensis*	KR922119	48
86	Marine	59b58	Actinobacteria	*Clavibacter michiganensis*	KR922179	48
54	Marine	32b56	Bacteroidetes	*Flavobacterium* sp.	HQ256854	48
29	Marine	13b03	ϒ-Proteobacteria	*Pseudomonas graminis*	DQ512786	48
54	Marine	32b55	ϒ-Proteobacteria	*Pseudomonas graminis*	HQ256853	48
50	Marine	30b02	Actinobacteria	*Rhodococcus* sp.	HQ256817	48
50	Marine	30b05	Actinobacteria	*Rhodococcus* sp.	HQ256819	48
84	Marine	57b11	α-Proteobacteria	*Sphingomonas* sp.	KR922112	48
49	Marine	29b04	Basidiomycota	*Sporobolomyces roseus*	HQ256893	48
49	Marine	29b05	Basidiomycota	*Udeniomyces pannonicus*	HQ256894	48
21	Marine	05b02	Firmicutes	*Bacillus* sp.		49
47	Marine	28b04	Basidiomycota	*Bensingtonia yucciola*	HQ256887	49
54	Marine	32b09	Basidiomycota	*Dioszegia hungarica*	HQ256898	49
75	Marine	48b10	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922063	49
86	Marine	59b15	ϒ-Proteobacteria	*Pseudomonas syringae*	KR922152	49
84	Marine	57b16	Actinobacteria	*Clavibacter michiganensis*	KR922117	50
86	Marine	59b50	Actinobacteria	*Clavibacter* sp.	KR922173	50
87	Marine	60b04	Actinobacteria	*Frigoribacterium* sp.	KR922183	50
85	Marine	58b20	ϒ-Proteobacteria	*Pseudomonas graminis*	KR922133	50
86	Marine	59b01	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922140	50
86	Marine	59b06	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922144	50
86	Marine	59b17	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922154	50
86	Marine	59b40	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922169	50
89	Marine	63b11	Actinobacteria	*Clavibacter* sp.	KR922218	51
85	Marine	58b05	Actinobacteria	*Curtobacterium* sp.	KR922127	51
86	Marine	59b26	ϒ-Proteobacteria	*Dyella* sp.	KR922158	51
75	Marine	48b07	Actinobacteria	*Frigoribacterium* sp.	KR922060	51
91	Marine	66b01	ϒ-Proteobacteria	*Pseudomonas syringae*	KR922246	51
84	Marine	57b28	α-Proteobacteria	*Sphingomonas* sp.	KR922123	51
47	Marine	28b10	Basidiomycota	*Udeniomyces pannonicus*	HQ256881	51
84	Marine	57b10	Firmicutes	*Bacillus* sp.	KR922111	52
86	Marine	59b30	Actinobacteria	*Clavibacter michiganensis*	KR922161	52
54	Marine	32b35	Actinobacteria	*Frigoribacterium* sp.	HQ256839	52
86	Marine	59b34	Actinobacteria	*Frigoribacterium* sp.	KR922164	52
86	Marine	59b57	Bacteroidetes	*Pedobacter agri*	KR922178	52
85	Marine	58b21	Bacteroidetes	*Pedobacter* sp.	KR922134	52
91	Marine	66b03	Bacteroidetes	*Pedobacter* sp.	KR922248	52
86	Marine	59b38	ϒ-Proteobacteria	*Pseudomonas rhizosphaerae*	KR922167	52
86	Marine	59b29	Actinobacteria	*Rhodococcus* sp.		52
84	Marine	57b13	α-Proteobacteria	*Sphingomonas* sp.	KR922114	52
84	Marine	57b07	unidentified bacteria	*unidentified*		52
89	Marine	63b09	Actinobacteria	*Clavibacter michiganensis*	KR922216	53
84	Marine	57b12	Actinobacteria	*Curtobacterium* sp.	KR922113	53
55	Marine	33b11	Basidiomycota	*Dioszegia hungarica*	JF706591	53
50	Marine	30b01	Actinobacteria	*Frigoribacterium* sp.	HQ256816	53
82	Marine	55b02	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922096	53
86	Marine	59b53	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922175	53
47	Marine	28b15	Basidiomycota	*Rhodotorula* sp.	HQ256885	53
55	Marine	33b12	α-Proteobacteria	*Sphingomonas* sp.	HQ256874	53
87	Marine	60b31	α-Proteobacteria	*Sphingomonas* sp.	KR922202	53
97	Marine	67b09	α-Proteobacteria	*Sphingomonas* sp.	KR922258	53
97	Marine	67b28	Actinobacteria	*Clavibacter michiganensis*	KR922268	54
47	Marine	28b03	Basidiomycota	*Cryptococcus victoriae*	HQ256886	54
85	Marine	58b17	Actinobacteria	*Curtobacterium* sp.		54
86	Marine	59b28	ϒ-Proteobacteria	*Dyella* sp.	KR922160	54
85	Marine	58b04	Actinobacteria	*Frigoribacterium* sp.	KR922126	54
89	Marine	63b02	Actinobacteria	*Frigoribacterium* sp.	KR922209	54
86	Marine	59b08	ϒ-Proteobacteria	*Pseudomonas fluorescens*	KR922146	54
54	Marine	32b66	ϒ-Proteobacteria	*Pseudomonas graminis*	HQ256863	54
75	Marine	48b11	α-Proteobacteria	*Sphingomonas* sp.	KR922064	54
81	Marine	54b07	α-Proteobacteria	*Sphingomonas* sp.	KR922094	54
87	Marine	60b16	α-Proteobacteria	*Sphingomonas* sp.	KR922193	54
87	Marine	60b22	α-Proteobacteria	*Sphingomonas* sp.	KR922195	54
97	Marine	67b22	Basidiomycota	*Udeniomyces pannonicus*	KR922311	54
54	Marine	32b05	Basidiomycota	*Udeniomyces* sp.	HQ256897	54
49	Marine	29b03	Firmicutes	*Bacillus* sp.	JF706502	55
54	Marine	32b13	Actinobacteria	*Clavibacter* sp.	HQ256832	55
97	Marine	67b30	Actinobacteria	*Clavibacter* sp.	KR922269	55
86	Marine	59b27	ϒ-Proteobacteria	*Dyella* sp.	KR922159	55
75	Marine	48b09	ϒ-Proteobacteria	*Pantoe* sp.	KR922062	55

87	Marine	60b12	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922190	55
89	Marine	63b30	ϒ-Proteobacteria	*Pseudomonas* sp.	KR922230	55
49	Marine	29b02	Actinobacteria	*Rhodococcus* sp.	HQ256815	55
87	Marine	60b23	α-Proteobacteria	*Sphingomonas* sp.	KR922196	55
54	Marine	32b21	Basidiomycota	*Udeniomyces* sp.	JF706580	55
97	Marine	67b25	Basidiomycota	*Udeniomyces* sp.	KR922312	55
49	Marine	29b01	Basidiomycota	*Bensingtonia yucciola*	HQ256892	56
87	Marine	60b03	α-Proteobacteria	*Brevundimonas* sp.	KR922182	56
71	Marine	46b09	Actinobacteria	*Curtobacterium* sp.		56
54	Marine	32b58	ϒ-Proteobacteria	*Ewingella americana*	JF706505	56
84	Marine	57b14	Actinobacteria	*Frigoribacterium* sp.	KR922115	56
86	Marine	59b54	Actinobacteria	*Frigoribacterium* sp.	KR922176	56
86	Marine	59b44	ϒ-Proteobacteria	*Pantoe agglomerans*	KR922172	56
54	Marine	32b60	ϒ-Proteobacteria	*Pseudomonas graminis*	HQ256858	56
86	Marine	59b42	α-Proteobacteria	*Sphingomonas* sp.	KR922171	56
54	Marine	32b12	Basidiomycota	*Udeniomyces pannonicus*	JF706578	56
85	Marine	58b23	Bacteroidetes	*Epiltholimonas sp.*		57
89	Marine	63b39	Bacteroidetes	*Pedobacter* sp.	KR922237	57
66	Marine	41b02	Actinobacteria	*Rhodococcus* sp.	JF706542	57
97	Marine	67b23	α-Proteobacteria	*Sphingomonas* sp.	KR922265	57
89	Marine	63b42	Actinobacteria	*Subtercola* sp.	KR922240	57
54	Marine	32b02	Actinobacteria	*Agreia* sp.	HQ256834	58
71	Marine	46b08	Actinobacteria	*Curtobacterium* sp.	KR922054	58
89	Marine	63b08	Actinobacteria	*Frondihabitans* sp.	KR922215	58
85	Marine	58b22	Bacteroidetes	*Pedobacter* sp.	KR922135	58
50	Marine	30b06	Actinobacteria	*Rhodococcus* sp.	HQ256820	58
79	Marine	52b02	Actinobacteria	*Rhodococcus* sp.	KR922085	58
87	Marine	60b06	α-Proteobacteria	*Sphingomonas* sp.	KR922184	58
87	Marine	60b11	α-Proteobacteria	*Sphingomonas* sp.	KR922189	58
89	Marine	63b43	α-Proteobacteria	*Sphingomonas* sp.	KR922241	58
85	Marine	58b06	unidentified bacteria	*unidentified*		58
87	Marine	60b10	ϒ-Proteobacteria	*Xanthomonas* sp.	KR922188	58
50	Marine	30b04	Actinobacteria	*Agreia* sp.	HQ256818	59
84	Marine	57b27	Actinobacteria	*Clavibacter michiganensis*	KR922122	59
89	Marine	63b38	Actinobacteria	*Clavibacter michiganensis*	KR922236	59
84	Marine	57b03	Actinobacteria	*Clavibacter* sp.	KR922107	59
97	Marine	67b17	Actinobacteria	*Nocardioides* sp.		59
97	Marine	67b19	ϒ-Proteobacteria	*Pseudomonas* sp.		59
97	Marine	67b29	Actinobacteria	*Rhodococcus* sp.		59
86	Marine	59b39	α-Proteobacteria	*Sphingomonas* sp.	KR922168	59
87	Marine	60b32	α-Proteobacteria	*Sphingomonas* sp.	KR922203	59
54	Marine	32b68	Basidiomycota	*Udeniomyces* sp.	JF706587	59
97	Marine	67b04	Firmicutes	*Bacillus* sp.	KR922255	60
86	Marine	59b20	α-Proteobacteria	*Brevundimonas* sp.	KR922156	60
87	Marine	60b15	α-Proteobacteria	*Brevundimonas* sp.		60
47	Marine	28b14	β-Proteobacteria	*Burkholderia* sp.	HQ256814	60
81	Marine	54b02	Actinobacteria	*Clavibacter michiganensis*	KR922091	60
47	Marine	28b07	Basidiomycota	*Cryptococcus* sp.	HQ256890	60
89	Marine	63b03	Actinobacteria	*Frigoribacterium* sp.	KR922210	60
54	Marine	32b61	α-Proteobacteria	*Sphingomonas* sp.	HQ256859	60
89	Marine	63b40	α-Proteobacteria	*Sphingomonas* sp.	KR922238	60
54	Marine	32b40	Basidiomycota	*Udeniomyces* sp.	JF706582	60
86	Marine	59b33	Firmicutes	*Bacillus* sp.	KR922163	61
47	Marine	28b13	Basidiomycota	*Cryptococcus* sp.	HQ256884	61
85	Marine	58b16	Actinobacteria	*Curtobacterium* sp.	KR922130	61
54	Marine	32b59	ϒ-Proteobacteria	*Ewingella americana*	HQ256856	61
54	Marine	32b63	Actinobacteria	*Plantibacter* sp.	HQ256861	61
54	Marine	32b03	Actinobacteria	*Rathayibacter* sp.	JF706503	61
87	Marine	60b07	α-Proteobacteria	*Sphingomonas* sp.	KR922185	61
87	Marine	60b30	α-Proteobacteria	*Sphingomonas* sp.	KR922201	61
89	Marine	63b41	Actinobacteria	*Subtercola* sp.	KR922239	61
54	Marine	32b65	α-Proteobacteria	*Agrobacterium* sp.	HQ256862	62
97	Marine	67b21	Basidiomycota	*Cryptococcus* sp.	KR922310	62
54	Marine	32b36	Actinobacteria	*Curtobacterium* sp.	HQ256840	62
47	Marine	28b05	Basidiomycota	*Dioszegia crocea*	HQ256888	62
54	Marine	32b33	ϒ-Proteobacteria	*Pseudoxanthomonas* sp.	HQ256838	62
54	Marine	32b45	α-Proteobacteria	*Sphingomonas* sp.	HQ256844	62
54	Marine	32b50	α-Proteobacteria	*Sphingomonas* sp.	HQ256848	62
55	Marine	33b06	Basidiomycota	*Udeniomyces* sp.	JF706589	62
86	Marine	59b55	β-Proteobacteria	*Variovorax paradoxus*	KR922177	62
89	Marine	63b05	Actinobacteria	*Arthrobacter nicotinovorans*	KR922212	63

97	Marine	67b08	Basidiomycota	*Cryptococcus* sp.	KR922308	63
55	Marine	33b07	Basidiomycota	*Cryptococcus victoriae*	JF706590	63
86	Marine	59b36	Actinobacteria	*Curtobacterium* sp.	KR922165	63
87	Marine	60b28	Basidiomycota	*Dioszegia zslotii*		63
89	Marine	63b19	Actinobacteria	*Frigoribacterium* sp.	KR922223	63
54	Marine	32b31	ϒ-Proteobacteria	*Pseudomonas syringae*	JN176585	63
86	Marine	59b51	Actinobacteria	*Rathayibacter* sp.	KR922174	63
87	Marine	60b29	α-Proteobacteria	*Sphingomonas* sp.	KR922200	63
89	Marine	63b15	α-Proteobacteria	*Sphingomonas* sp.	KR922221	63
91	Marine	66b11	α-Proteobacteria	*Sphingomonas* sp.	KR922250	63
97	Marine	67b20	Basidiomycota	*Udeniomyces pannonicus*	KR922309	63
85	Marine	58b15	β-Proteobacteria	*Variovorax* sp.	KR922129	63
75	Marine	48b04	Bacteroidetes	*Chryseobacterium* sp.	KR922058	64
89	Marine	63b20	Actinobacteria	*Clavibacter michiganensis*	KR922224	64
89	Marine	63b47	Actinobacteria	*Clavibacter michiganensis*	KR922244	64
47	Marine	28b06	Basidiomycota	*Dioszegia crocea*	HQ256889	64
54	Marine	32b15	Basidiomycota	*Dioszegia* sp.	JF706579	64
54	Marine	32b17	Actinobacteria	*Frigoribacterium* sp.	HQ256833	64
50	Marine	30b07	ϒ-Proteobacteria	*Psychrobacter sp.*	HQ256821	64
54	Marine	32b29	α-Proteobacteria	*Rhizobium* sp.	HQ256837	64
54	Marine	32b11	α-Proteobacteria	*Sphingomonas* sp.	HQ256831	64
87	Marine	60b13	α-Proteobacteria	*Sphingomonas* sp.	KR922191	64
89	Marine	63b10	α-Proteobacteria	*Sphingomonas* sp.	KR922217	64
89	Marine	63b27	α-Proteobacteria	*Sphingomonas* sp.	KR922229	64
89	Marine	63b36	α-Proteobacteria	*Sphingomonas* sp.	KR922234	64
54	Marine	32b14	unidentified bacteria	*unidentified*		64
89	Marine	63b21	β-Proteobacteria	*Variovorax* sp.	KR922225	64
32	Marine	16b01	Firmicutes	*Bacillus* sp.		65
97	Marine	67b11	α-Proteobacteria	*Brevundimonas* sp.	KR922259	65
89	Marine	63b34	Bacteroidetes	*Chryseobacterium* sp.		65
47	Marine	28b01	Basidiomycota	*Dioszegia crocea*	HQ260319	65
54	Marine	32b41	Basidiomycota	*Dioszegia* sp.	JF706583	65
54	Marine	32b28	Actinobacteria	*Frondihabitans* sp.	HQ256836	65
80	Marine	53b01	ϒ-Proteobacteria	*Moraxella* sp.	KR922089	65
97	Marine	67b16	Actinobacteria	*Promicromonospora sp.*	KR922263	65
84	Marine	57b25	α-Proteobacteria	*Sphingomonas* sp.	KR922120	65
86	Marine	59b19	α-Proteobacteria	*Sphingomonas* sp.	KR922155	65
54	Marine	32b51	Actinobacteria	*Subtercola* sp.	HQ256849	65
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54	Marine	32b46	Actinobacteria	*Curtobacterium herbarum*	HQ256845	66
54	Marine	32b73	Basidiomycota	*Dioszegia fristigensis*	JN176595	66
54	Marine	32b37	Basidiomycota	*Dioszegia* sp.	JF706581	66
87	Marine	60b27	β-Proteobacteria	*Duganella* sp.	KR922199	66
85	Marine	58b27	Bacteroidetes	*Flavobacterium* sp.	KR922138	66
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89	Marine	63b13	β-Proteobacteria	*Janthinobacterium* sp.	KR922220	66
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81	Marine	54b03	Actinobacteria	*Rhodococcus* sp.	KR922092	66
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84	Marine	57b15	α-Proteobacteria	*Sphingomonas* sp.	KR922116	66
97	Marine	67b13	Actinobacteria	*Streptomyces* sp.	KR922260	66
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85	Marine	58b07	Bacteroidetes	*Epiltholimonas sp.*		67
97	Marine	67b15	Actinobacteria	*Janibacter* sp.	KR922262	67
54	Marine	32b54	Actinobacteria	*Rathayibacter* sp.	HQ256852	67
97	Marine	67b07	α-Proteobacteria	*Rhizobium* sp.	KR922257	67
84	Marine	57b04	α-Proteobacteria	*Sphingomonas* sp.	KR922108	67
87	Marine	60b20	α-Proteobacteria	*Sphingomonas* sp.		67
89	Marine	63b35	α-Proteobacteria	*Sphingomonas* sp.	KR922233	67
89	Marine	63b45	α-Proteobacteria	*Sphingomonas* sp.	KR922242	67
97	Marine	67b24	α-Proteobacteria	*Sphingomonas* sp.	KR922266	67
54	Marine	32b71	Basidiomycota	*Udeniomyces puniceus*	JN176594	67
97	Marine	67b32	Unidentified yeast	*unidentified*		67
72	Marine	47b02	β-Proteobacteria	*Variovorax* sp.	KR922055	67
54	Marine	32b48	Basidiomycota	*Bullera globispora*	JF706585	68
85	Marine	58b19	Bacteroidetes	*Hymenobacter* sp.	KR922132	68
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79	Marine	52b08	α-Proteobacteria	*Sphingomonas* sp.	KR922088	68
87	Marine	60b08	α-Proteobacteria	*Sphingomonas* sp.	KR922186	68

87	Marine	60b09	α-Proteobacteria	*Sphingomonas* sp.	KR922187	68
89	Marine	63b04	α-Proteobacteria	*Sphingomonas* sp.	KR922211	68
89	Marine	63b37	α-Proteobacteria	*Sphingomonas* sp.	KR922235	68
54	Marine	32b47	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256846	68
55	Marine	33b05	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256869	68
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72	Marine	47b05	Bacteroidetes	*Hymenobacter* sp.	KR922056	69
91	Marine	67b01	β-Proteobacteria	*Janthinobacterium* sp.	KR922252	69
62	Marine	37b05	Ɣ-Proteobacteria	*Pseudomonas fluorescens*	JF706535	69
78	Marine	51b08	Ɣ-Proteobacteria	*Pseudomonas* sp.	KR922083	69
81	Marine	54b04	α-Proteobacteria	*Sphingomonas* sp.		69
81	Marine	54b06	α-Proteobacteria	*Sphingomonas* sp.	KR922093	69
84	Marine	57b02	α-Proteobacteria	*Sphingomonas* sp.	KR922106	69
84	Marine	57b08	α-Proteobacteria	*Sphingomonas* sp.	KR922110	69
89	Marine	63b07	α-Proteobacteria	*Sphingomonas* sp.	KR922214	69
89	Marine	63b12	α-Proteobacteria	*Sphingomonas* sp.	KR922219	69
89	Marine	63b26	α-Proteobacteria	*Sphingomonas* sp.	KR922228	69
89	Marine	63b33	α-Proteobacteria	*Sphingomonas* sp.	KR922232	69
97	Marine	67b03	α-Proteobacteria	*Sphingomonas* sp.	KR922254	69
97	Marine	67b14	Firmicutes	*Staphylococcus xylosus*	KR922261	69
79	Marine	52b05	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	KR922087	69
97	Marine	67b06	Actinobacteria	*Cellulomonas cellasea*	KR922256	70
87	Marine	60b25	β-Proteobacteria	*Massilia* sp.		70
80	Marine	53b06	Actinobacteria	*Nocardioides* sp.	KR922090	70
54	Marine	32b49	α-Proteobacteria	*Sphingomonas* sp.	HQ256847	70
75	Marine	48b08	α-Proteobacteria	*Sphingomonas* sp.	KR922061	70
79	Marine	52b03	α-Proteobacteria	*Sphingomonas* sp.	KR922086	70
81	Marine	54b05	α-Proteobacteria	*Sphingomonas* sp.		70
87	Marine	60b19	α-Proteobacteria	*Sphingomonas* sp.	KR922194	70
97	Marine	67b26	α-Proteobacteria	*Sphingomonas* sp.	KR922267	70
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54	Marine	32b43	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256843	70
54	Marine	32b72	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256871	70
55	Marine	33b03	Ɣ-Proteobacteria	*Xanthomonas campestris*	HQ256868	70
87	Marine	60b02	Ɣ-Proteobacteria	*Xanthomonas* sp.	KR922181	70
54	Marine	32b06	Bacteroidetes	*Flavobacterium* sp.	HQ256857	71
81	Marine	54b11	Bacteroidetes	*Hymenobacter* sp.	KR922095	71
54	Marine	32b57	α-Proteobacteria	*Sphingomonas* sp.	HQ256855	71
55	Marine	33b09	α-Proteobacteria	*Sphingomonas* sp.	HQ256873	71
72	Marine	47b03	α-Proteobacteria	*Sphingomonas* sp.		71
87	Marine	60b33	α-Proteobacteria	*Sphingomonas* sp.	KR922204	71
54	Marine	32b69	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256865	71
55	Marine	33b01	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256866	71
79	Marine	52b07	Ɣ-Proteobacteria	*Stenotrophomonas* sp.		71
87	Marine	60b14	α-Proteobacteria	*Brevundimonas* sp.	KR922192	72
97	Marine	67b02	Bacteroidetes	*Chryseobacterium* sp.	KR922253	72
47	Marine	28b09	Basidiomycota	*Dioszegia hungarica*	HQ256891	72
84	Marine	57b18	Bacteroidetes	*Flavobacterium* sp.	KR922118	72
54	Marine	32b04	α-Proteobacteria	*Sphingomonas* sp.	HQ256841	72
54	Marine	32b39	α-Proteobacteria	*Sphingomonas* sp.	JF706504	72
54	Marine	32b30	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	JN176584	72
54	Marine	32b38	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	JN176587	72
55	Marine	33b08	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	HQ256870	72
23	Polluted	07b11	Firmicutes	*Bacillus* sp.		31
53	Polluted	31b07	Actinobacteria	*Rhodococcus* sp.	HQ256828	50
53	Polluted	31b08	Actinobacteria	*Frigoribacterium* sp.	HQ256829	52
88	Polluted	61b06	Ɣ-Proteobacteria	*Stenotrophomonas* sp.	KR922206	54
53	Polluted	31b04	Ɣ-Proteobacteria	*Pseudomonas* sp.	HQ256826	56
53	Polluted	31b03	Actinobacteria	*Kocuria palustris*	HQ256825	60
23	Polluted	07b06	Firmicutes	*Paenibcillus* sp.		60
53	Polluted	31b02	Actinobacteria	*Rhodococcus* sp.	HQ256824	62
88	Polluted	61b10	Bacteroidetes	*Chryseobacterium* sp.	KR922207	66
53	Polluted	31b01	Firmicutes	*Bacillus megaterium*	HQ256822	68
53	Polluted	31b09	Actinobacteria	*Streptomyces* sp.	HQ256830	68
53	Polluted	31b10	β-Proteobacteria	*Massilia* sp.	HQ256823	71

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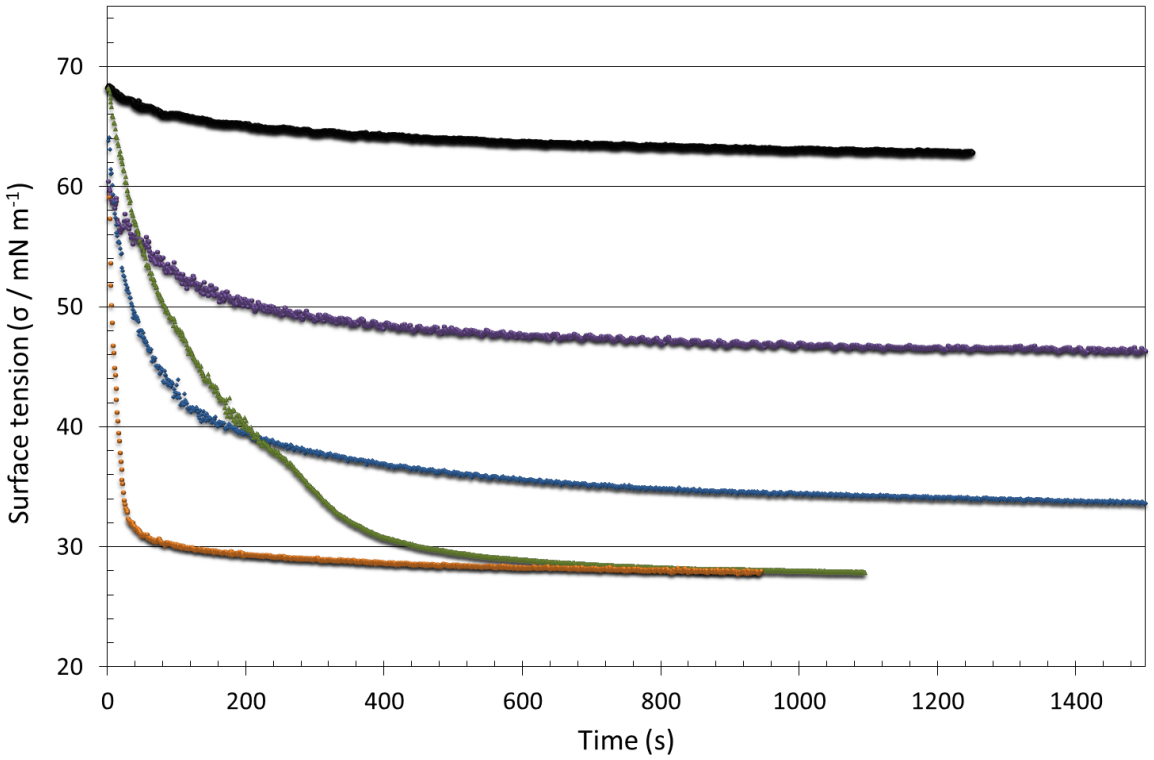

Figure 2. Time profile of surface tension measurements in a non-logarithmic form.

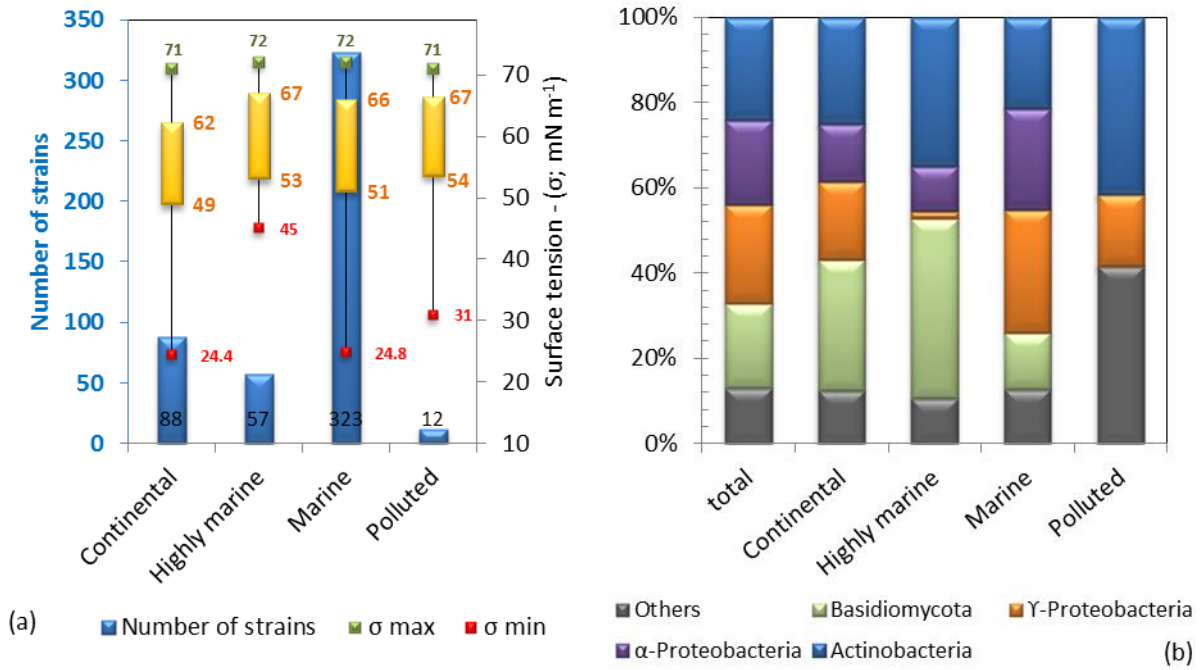

Figure 4 (a). Surface tension (σ) distribution of the 480 strains tested for biosurfactant production according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted). Highlighted in blue, the number of tested strains. Box and whisker plots are shown 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).

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Screening of cloud microorganisms isolated at the puy de Dôme (France) station for the production of biosurfactants

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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 (σ_{eq}), 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, t_{eq}, is of the order of $2 \times t_m$ (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 (σ_0), the surface tensions in the meso-equilibrium (σ_m) and in the equilibrium (σ_{eq}) 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 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, 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).

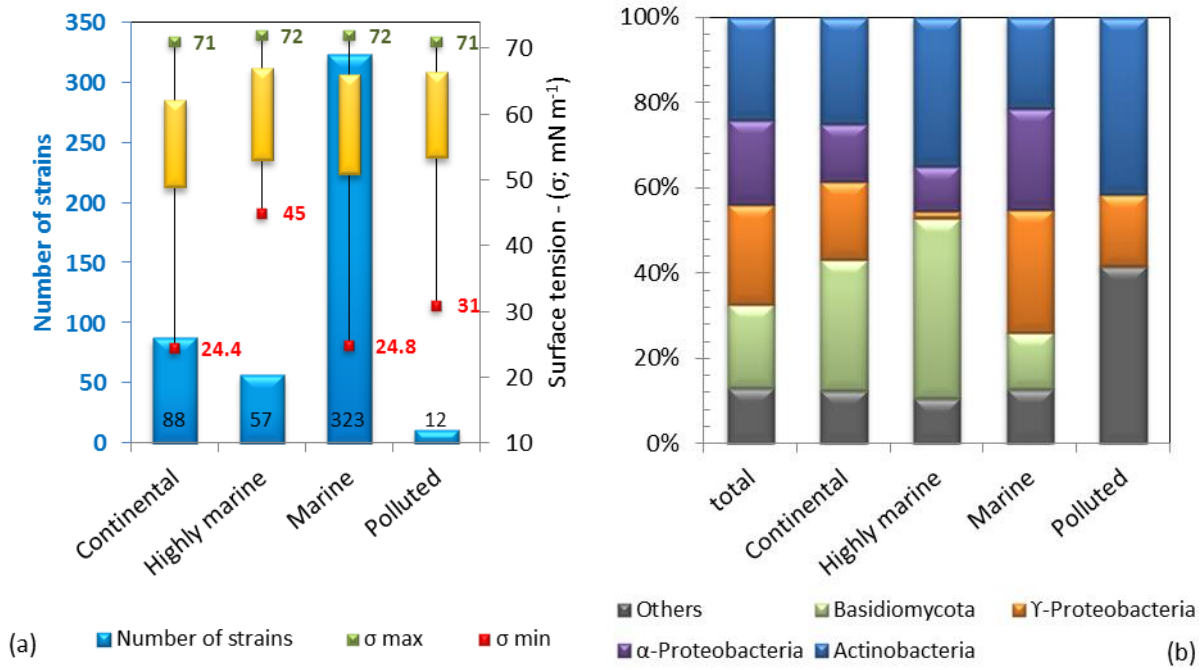

Figure 4 (a). Surface tension (σ) distribution of the 480 strains examined for biosurfactant production according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted). Highlighted in blue, the number of tested strains. Box and whisker plots are shown 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 ϒ-Proteobacteria, which are the most efficient biosurfactant-producing microorganisms (σ ≤ 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.

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)".

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 #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 ϒ-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 (~ 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.".*

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* (ϒ-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 ϒ-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 $\sigma > 55$ 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, SouthWest/West).

2) The chemical content of cloud water: The physicochemical parameters presented in **Table S1** (pH, SO_4^{2-}, NO_3^-, Cl^-, Acetate, Formate, Oxalate, Succinate, Malonate, Na^+, NH_4^+, Mg^{2+}, K^+,Ca^{2+}) 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 NH_4^+, NO_3^-, SO_4^{2-}. 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 Ɣ-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 ϒ-Proteobacteria class (Fig. 6). In the NW/N sector, most efficient biosurfactant-producing microorganisms account for 68% of ϒ-Proteobacteria (13/19 isolates), against 40% in the other three sectors (37/93 isolates). No such difference amongst the ϒ-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, 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)."

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, 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).

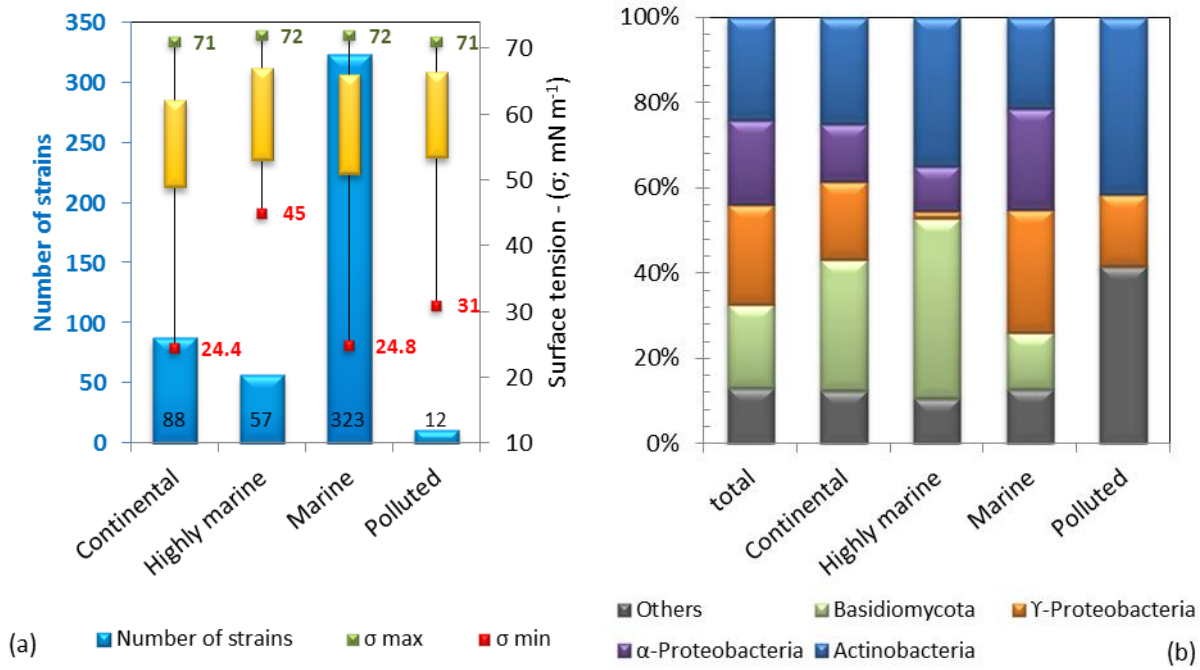

Figure 4 (a). Surface tension (σ) distribution of the 480 strains examined for biosurfactant production according to the physicochemical characteristics of cloud waters (marine, highly marine, continental and polluted). Highlighted in blue, the number of tested strains. Box and whisker plots are shown 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 ϒ-Proteobacteria, which are the most efficient biosurfactant-producing microorganisms (σ ≤ 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 ($PM_{1\text{-}2.5}$) reach lung alveoli and ultrafine particles ($PM_{0.1}$), 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; DIFCO™), and eventually onto R2A medium supplemented with NaCl 20 g L^{-1} and King's B (King et al., 1954), Sabouraud (DIFCO™) and TSA (Trycase Soy Agar, DIFCO™) 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