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Interactive comment on "Potential for phenol biodegradation in cloud water" *by* Audrey Lallement et al.

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General comments The study of the potential biodegradation of a major pollutant, phenol, by bacteria in cloud water presented in this paper is a pioneer work that is very important for understanding the global cycle of some toxic components and the activity of microorganisms in atmosphere. The use of both molecular and cuturable approaches is very convincing and these approaches are complementary. Metatranscriptomic analysis and biodegradation tests showed clearly the potentiality of phenol biodegradation in atmosphere and open question about the phenol biodegradation rates under realistic cloud conditions. The use of different cloud sampling for the different analyses (phenol quantification, metatranscriptomic analysis and phenol degradation tests) has to be better justified and taken into account in the discussion. Experimental design of the

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biodegradation test has to be better explain. The distinction between the results from molecular versus culturable approaches need sometimes to be clarify. Major changes have to be done in the discussion about P. syringae strains.

Detailed comments Abstract : L 24 : Concentration of phenol in cloud samples was measured only in 2 samples in this work. The three other values are from a previous paper. Clarify. L 27 : Work has been done on strains not on isolates. Specify that the strains were isolated in a previous work. L 28 : Details on Puy station should be placed L 25 L 29-30 : Specify that the 3 samples were different of those used for phenol quantification L 35 : Specify that strains were selected in species known for having this activity

Introduction L 56 : Phenol and 4-ethylphenol are the most abundant phenols in clouds (Lebedev et al 2018, Table 2). Why have you limited the study to Phenol ? Are they degraded by the same enzymes? L 69-71 & 119-121 : It may be possible to decrease the reference number and keep the most significant L 123 : Add “in clouds” before the references L 126 : Metatranscriptomic allowed to detect gene expression (transcripts), it is more than simply detecting genes.

Materials & methods L 142-144 : 3 of the 5 samples of cloud water were extracted from Lebedev et al.2018 and only 2 were done in this study. Clarify. L 166-167 : Doing metatranscriptomic and phenol quantification on different samples must be better justify. Why choosing 3 consecutive periods of 5 h for the 3 samples for metatranscriptomic (are they considered as replicates ?), rather than 3 independent samples (different dates) ? It should be informative to have Hysplit information and phenol concentration for these 3 samples. L 169-172 : This control is great. Add some information on the transcriptomic result of it. L 188 : I think that it is Figure 1 that content information and not Figure 2. 189-190 : In these databases, have you included the “catechol operon” cited in Berge et al. 2014 that you compare to your data in the discussion (see details comments of the discussion below). Do these enzymes could be involved in other activities than phenol and catechol degradation? L 199-200 : Specify that strains

were isolated previously from different samples L 201 : The sampling of bacteria is not randomized why ? May be you should have found strains with degrading activity that you would'nt expected. The chosen strains expected to show an activity do not represent at all the cloud bacterial population. In the abstract you must explain that, before to give the percentage of positive strains for phenol biodegradation to not suggest that 93 % of cloud culturable bacteria are able to degrade phenol. L 201-207 : It should be great to know the abundance of these strains when isolated to have an idea of their importance in cloud (size of their population). L 204 : I expect that P. grimortii does not exist, check this name. Table SM1 : Specify that accession number is for 16S RNA gene sequence L 208 : In which volume were done the initial bacterial cultures ? L210 : Does Volvic water sterilized ? L212-218 : This section need to be better explained. It is not clear how bacterial concentrations were measured and when. Why have you chosen the x 104 factor ? 109 cells/ml seems to be very high bacterial concentration, justify. L 226-228 : Better to transfer this section in result or discussion: (it is already repeated L 311 -316) L 248-249 : What is the experimental design of this test ? Have you replicate the test ? If not, why ?

Result L 252 : previously isolated Table 1 : Usually, table have to be in column, with title in the first line. Unit could be in the title. L 265 : I think that you must cite Figure SM2 C, D, E in the text. L 266 : I should have write "Microbiote" L 272 :Table SM2, do not contain P. syringae sequences why (see related comments in the discussion part) ? L 275-277 : does this variability could be explain by various probabilities that a given degrading bacterial population encounter a given amount of phenol in cloud droplets ? This question has to be discussed somewhere. L 278-281 : Are these differences significant ? L283-285 : Is it the microbial activity or the microbial diversity that varied ? Is it in time or in space in the cloud ? This comment need to be clearer. L 286 : Figure 3 not Figure 2 L 290 : matching not matched. 2 sentences would be clearer. L292 : were tested ? were found in data bases L 288-299 : English has to be improved, to facilitate the understanding L295 : Clarify: which approach was used to calculate this 0.3 % ? Was it on the same samples studied in the metatranscriptomic analysis ?

L298 : Explain better why referring to Rhodococcus in this section. L 302 : culturable L 304-305 : Let us know how were selected the genus of interest ? From literature knowledge ? In comparison with table SM2 ? Anything else ? Figure 4 : why have you chosen to test many strains without replicate, when you may have chosen less strains with replication of the test? Actually, we have no idea of the test variability for one given strain. L 320 : “genus” not “strains” L 320 – 327 : Figure 4 B : Some degrading species of Pseudomonas are not present in databases used for bioinformatics. Genomes have been sequenced in some strains of these species, is it possible to find genes involved in phenol degradation in these genomes? L 325 : Reword this line : P. syringae is a species name not a genus. Which approach showed that P. syringae is the most abundant bacterial species in cloud water ?

Discussion & Conclusion: L 332 : Citation of Lebedev et al. 18 as a comparison is may not correct, when some data came from this paper L 333 : Add the range of phenol concentration found in all these papers (3.0 to 5.4 mgL-1 ?) L333-335 : Compare your data with those of Lebedev et al 2018. They found in the results section: “no major impact of the air mass origin” “The anthropization of the air masses seems to increase the levels of phenol and 4-nitrophenol in the clouds (our work and the literature)” To test the effect of air masses origins, you have two replicates of west origin and two from north west/north, which could be statistically compared. It will probably show, it is those from the non-polluted area that have the higher concentrations. Comment. L 335 : “Slight variation”: I would say rather “in the same magnitude” because concentrations from West are approx. x 3 those of North west/North L 338: Not enzymes were detected, their trancripts were. L339: Sequences not species. Explain why referring here to Rhodococcus. You should say that you didn’t find all the other bacterial species present in the data base (Table SM2) L 339: Replace “in parallel” by something like : “Culturable approach has shown previously than Rodoc and Pseudo were abundant but etc. . .(cite the papers)” L 343: Database constitution: what could you propose to improve the data base for phenol degradation ? You have tested strains of species that were not in data base and that showed phenol degradation. L 344: Culturable L

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344: Aman et al. 1995 citation could be replaced if possible by a more recent one, containing estimations of percent of culturable bacteria done on cloud water or on substrates close to it. If you keep Aman et al. 95, percent of culturable bacteria in water were lower than 1 % if I well remember, check this. L 345-346: Better to say "strains from Acinetobacter, Pseudomonas and Rhodoccoccus genera are known to degrade phenol. . ." L 347 : Microbiote not microbiome. Specify if you speak about the microbiote described from the culturable or molecular approaches L 351: "Actinobacteria" is not useful here L 362-364: If possible cite only the main references reporting the range of values in surface water. L 365: Pseudomonas are more frequent in culturable bacteria of cloud water but not in metagenomic analysis. L 366-367: If P. syringae strains are able to degrade phenol (and may be other strains) why we don't find them in your data bases of phenol degradative enzymes (Table SM2) ? See related comments below on the ref : Bartoli et al. 2015. L 366-367 : Reformulate the sentence : not only P. syringae and P. graminis could be issued from phyllosphere. P. fluorescens, P. poae, P. viridiflava could also. L 369-382 and Table SM3 : Actually data from Berge et al 2014, are not pertinent for comparison with this study because the 763 strains studied in Berge et al. represented a very wide diversity (more than 20 potential species, see Gomila et al. 2017) when strains from clouds may represent less diversity. May be it would be possible to compare strains from clouds with those from other environments, clade by clade that would suppose to determine the exact phylogroup and clade classification of cloud strains. Concerning the catechol operon, Berge et al. said p 6: "Strains in this clade [clade 01b], as well as strains in phylogroup 3 [PG03], contain a catechol operon regrouping genes for degradation of aromatic compounds [32]". It means that only some strains and not all of them, had this catechol operon in their genome in these phylogroups. Therefore values of Table SM3 are note correct. The original data are reported in the ref 32 of the Berge et al (2014) paper. This ref (Bartoli et al 2015) will be more pertinent to cite in your study: it is shown that not all phylogroups were tested for the presence of catechol operon in their genome: only 19 strains from PG01 (14 positive), 4 strains from PG03 (3 positive) and one strain from

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PG02 (negative). Therefore, Table SM3 and the related comments have to be profoundly modified. In particular, Bartoli et al. 2015 stated P 138 : “Comparison of gene content between publicly available genomes of several P. syringae pathogens of woody plants with those of herbaceous plants revealed an operon with predicted function in the catechol pathway that was present only in pathogens of woody plants”. Again, why these sequences were not integrated in your database ? These authors also shown (p 137) that “All environmental strains [tested in the study] possessing an operon involved in the degradation of aromatic compounds via the catechol pathway grew endophytically and caused symptoms in kiwifruit vascular tissue”. Concerning ice nucleating activity it is interesting to analyse the frequency of INA positive strains among the phenol degradative strains of P. syringae. Again it is not possible to compare with the percentages found by Berge et al. because of the very big difference in strain diversity but may be comparison of percentages clade by clade would be possible. You may discuss on the potential consequences of finding bacteria such as P. syringae, in clouds that have the catechol degrading operon linked with pathogenicity on woody plants, in terms of epidemiology, dispersion of pathogens and emergence of plant diseases. Discussion on the role of INA in this dispersion could be added. Discuss also the potential reverse consequences, the presence (manipulate or not) on phyllosphere of such P. syringae (or other phylospheric population) having the catechol degradative pathway operon, their driven by ascending air movements into the clouds and their effect on the phenol degradation in clouds (to be linked to comments on remediation L 424-425) L373 : In Berge et al. (2014) it is PG I & III that have catechol operon in their genomes and not PG I and II. Anyway these lines commenting Table SM3 have to be modified (see comments above) L 375 : Ice nucleation activity is not restricted to P. syringae and could be found mainly in Gammaproteobacteria, more specifically in Pseudomonadaceae (P. syringae, P. fluorescens), Xanthomonadaceae and Enterobacteriaceae. Why the study of INA was restricted to P. syringae ? L 389 : How can you assert that “enzymatic equipment for phenol degradation is largely present” ? Many strains were active, but they were chosen among the species assumed to be able to exhibit this

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activity and there are no quantification of their abundance in cloud water, therefore, we have no idea of the real quantitative impact of these strains. L 412 focused L 416 why Rhodococcus when it was not found in the metatranscriptomic analysis ?

Technical comments Referring to previous work must be stated more clearly in the text. Words like, isolate, strain, species, genus have to be used in the good way. Italics for Latin names L 318 : two “.”

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