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A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms

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Abstract

The interactions between microbial and chemical contents of cloud water were investigated. First, we observe that the bulk cloud water solution provides a substantial environment where bacteria can develop significantly. Then, a total number of 60 microbial strains originating from seven distinct samples of cloud water and affiliated to various taxonomic groups were looked for their ability to degrade some of the main atmospheric carboxylic compounds: formate, acetate, lactate, succinate, formaldehyde and methanol. Biodegradation tests show that all these compounds can be transformed when used as single carbonaceous substrates, with activities depending on both the strain and the compound. The highest capacities of biodegradation are observed towards formaldehyde, formate and acetate, which are also the more concentrated compounds typically measured in cloud water. Hence, analyses by $^1$H NMR permitted to establish for instance that compounds like pyruvate or fumarate can be produced and released in the media in relation to the transformation of lactate or succinate. In addition, utilization of $^{13}$C labelled formaldehyde showed that it can be transformed through many metabolic pathways, similar to those induced by photochemistry and leading to the production of formate and/or methanol. These results suggest that microorganisms of cloud water can have various behaviours towards the chemical compounds present in the atmosphere: they can represent either a sink or source for organic carbon, and may have to be considered as actors of cloud chemistry.

1 Introduction

Up to the last decade, the existence of a living microbial biota in fog and cloud water has been demonstrated (Fuzzi et al., 1997; Bauer et al., 2002; Amato et al., 2005), and the evidence of a possible metabolic activity under the conditions of temperature encountered in clouds was also given. The primary production resulting from the multiplication of micro-organisms in clouds was estimated to reach 1 to 10 Tg C per year (Sattler et
During previous investigations, we isolated a large variety of bacterial and fungal micro-organisms from cloud water samples in which ATP concentration implied microbial activity (Amato, unpublished Ph.D thesis, Université Blaise Pascal, 2006). This activity could be supported by the presence of substrates like atmospheric organic compounds, the chemistry of which has become a subject of increasing interest with the growing problems of air pollution. As suggested by Ariya and Amyot (2004), the microbiological component of clouds could interfere with the chemical processes occurring in droplets and finally have a non negligible influence on the chemical composition of cloud water.

Organic compounds are present in all the compartments of the atmosphere, the gaseous and particulate phases (Chameides and Davis, 1983; Puxbaum et al., 1988; Grosjean, 1989; Kumar et al., 1996; Sellegri et al., 2003), rain water (Kieber et al., 1999; Kawamura et al., 2001) and also in cloud water on which we focus in this paper (Voisin et al., 2000; Fuzzi et al., 2002; van Pinxteren et al., 2005; Parazols et al., 2006). Carboxylic acids, originating from both anthropogenic and biogenic sources, predominate and represent between 10% and more than 70% of the total dissolved organic carbon contained in cloud water (Löflund et al., 2002; Marinoni et al., 2004). High levels in aldehyde concentrations are strongly linked to human activities (Granby et al., 1997) and to photochemistry (Riedel et al., 1999). Alcohols, though they are rarely measured due to analytical problems, have been detected in polluted fog water using nuclear magnetic resonance (NMR) (Suzuki et al., 1998). All these compounds are related to the oxidation of hydrocarbons, mainly emitted from anthropic activities. They are in addition strongly linked together by atmospheric chemistry. As an example in the case of C\textsubscript{1} compounds, the transformation of methanol to formaldehyde, formate and finally to CO\textsubscript{2} is catalyzed by free radicals produced by photochemical processes (Monod et al., 2000). For the moment, investigations concerning the capacity of such atmospheric compounds to be microbiologically transformed showed some very interesting potentialities (Ariya al., 2002; Amato et al., 2005), but they are limited to a very few micro-organisms, and a real overall picture is still not available. To contribute to fill
this lack, we present in this article results obtained from a large study, involving 60 mi-
5
crobial strains isolated from cloud water samples collected all along a period of almost
two years, described elsewhere in a recently published paper (Amato et al., 2007). Six
organic compounds have been considered, among which four carboxylic acids (three
monoacids (formate C_1, acetate C_2 and lactate C_3) and one diacid (succinate C_4)), an
aldehyde (formaldehyde C_1) and an alcohol (methanol C_1). An introducing observation
is presented, proving the capacity of micro-organisms to develop under the nutritive
conditions provided by cloud water.

2 Material and methods

2.1 Capacity of cloud water to act as a nutritive medium

A volume of cloud water sampled at the puy de Dôme summit as described in Am-
atto et al (2005) have been brought back to the laboratory for a further incubation at
17°C, under agitation (200 rpm). All precautions were taken to prevent contamination
during all the experiment. ATP concentration was measured by bioluminescence (lu-
ciferin/luciferase) all along a period of about four days, on triplicate volumes of 0.2 mL
sampled under sterile conditions. Reagents from a commercial kit (Biothema, ATP
Biomass kit) were used, and the measurement was made with a bioluminometer Bio-
counter M2500 (Lumac). In addition, total cell counts have been carried out by epi-
fluorescence microscopy at the beginning and at the end of the incubation, in tripli-
cates. Volumes of 7.5 mL were fixed with equal volumes of prefiltered 4–5% formalde-
hyde, incubated 20 min in the dark in the presence of 2.5 μg mL^{-1} of 4′-6-diamino-2-
phenylindole (DAPI), filtered (GTBP 0.2 μm, Millipore) and filters were finally dried and
mounted on slides to be observed. Cells present on random microscopic fields were
counted (objective x40 for fungal cells, then x100 under immersion for bacteria), using
a microscope Olympus BH-2.
2.2 Incubations of strains in the presence of organic compounds

For each isolated strain, liquid pure pre-culture was incubated at 17°C or 27°C in M200, TS (Biomerieux) or R2 (prepared according to the R2A Difco medium) broths. Pure cultures were then incubated under the same conditions and cells were harvested by centrifugation (4000 g, 15 min, 4°C) after 24 or 48 h of growth, twice rinsed with NaCl 0.8% and finally suspended in the test media containing one of the compounds to degrade. The test media were composed of cells originating from a volume of 25 mL of culture, for an OD$_{575nm}$ of 10, with addition of about 20 mM of sodium formate (Aldrich), lactic acid (mix of isomers L and D, approximately 70:30) (Touzard & Matignon), sodium succinate (Aldrich) or 2 mM of formaldehyde and 0.8 mM of methanol from a mixed aqueous solution of formaldehyde/methanol (Sigma 37 % v/v formaldehyde, stabilized with 15% v/v of methanol) in 0.1 M phosphate buffer at pH 7.0. The values of culture volume used and of substrate concentration were adjusted to keep constant the ratio between cell density and amount of the compound to degrade. For a given strain, all tests were performed using cells originating from the same culture flask, which was dispatched between the tests media. One single compound was present in each of the incubation media, except for L- and D-lactate and for formaldehyde/methanol. A blank constituted by cells and phosphate buffer was systematically made. Incubations were carried out in 100 mL Erlenmeyer's flasks containing 25 mL of test medium, under agitation (200 rpm), at 17°C or 27°C depending of the culture conditions previously used and corresponding to the best conditions of growth for the considered strain. Samples of about 1 mL were taken at the beginning of the incubation and after 24 h, centrifuged (12 000 g, 3 min) to discard the cell pellet and the supernatants were kept frozen (–40°C) until analysis.

2.3 Organic acids and methanol quantifications by $^1$H NMR

Supernatants from biodegradation test media were prepared for $^1$H NMR by mixing a volume of 450 $\mu$L of the sample with 50 $\mu$L of sodium tetra deuteriated trimethylsilyl
propionate (TSPd₄, Eurisotop) in solution in D₂O. The latter was used for locking and
shimming, while TSPd₄ constituted a reference for chemical shifts (0 ppm) and quantifi-
cation. Final volumes of 500 µl of prepared samples were put in 5 mm-diameter tubes
for NMR. Acquisition of spectra were made at 400.13 MHz, 21°C, on a Bruker Avance
400 spectrometer, by collection of 32 scans (90° pulse, 4789.27 Hz SW, 65.536 data
points, 6.84 min total acquisition time). Water signal was eliminated by presaturation,
and no filter was applied before Fourier transformation. Using Bruker software (X-Win
NMR), baseline was corrected before integration for quantification. The concentration
of metabolites was calculated as follow: 

\[ [m] = \frac{(9 * A_o * [TSPd_4])}{(b * A_{ref})} \]

where \([m]\) is the concentration of the compound to quantify, \(A_o\) is the area of \(m\) resonance, \(A_{ref}\) is
the area of TSPd₄ resonance, and 9 and \(b\) are respectively the numbers of protons of
TSPd₄, resonating at 0 ppm, and of \(m\).

2.4 Formaldehyde quantification

Formaldehyde is not detectable by \(^1\)H NMR due to its chemical shift of about 4.5 ppm,
masked by the signal of water. So a sensitive automatic analyser was used (Aero-
laser AL4021). The principle is based on the reaction of formaldehyde with acetylacet-
tone and ammoniac, resulting in the formation of the detected fluorochrome complex
(λ_{excitation} = 400 nm; λ_{emission} = 510 nm), for which light emission is directly proportional to
formaldehyde concentration.

2.5 Utilization of \(^{13}\)C labelled formaldehyde and analysis by \(^{13}\)C NMR

Incubation in the presence of \(^{13}\)C labelled formaldehyde (Eurisotop, solution at 20%
v/v) was also carried out for three strains at 17°C and 5°C, to follow the formaldehyde
metabolism and unambiguously identify formed compounds as metabolites. Incuba-
tions were carried out as previously exposed, and acquisitions of \(^{13}\)C NMR spectra
were performed at 100.62 MHz using the same 400 MHz spectrometer than for \(^1\)H
NMR. 512 scans were collected (90° pulse, 25125.63 Hz SW, 65.536 data points,
54.19 min total acquisition time), and spectra were treated using the X-Win NMR software. This method is not quantitative, and the analyser AL4021 and $^1$H NMR have been used to quantify formaldehyde and formed labelled metabolites.

3 Results and discussion

3.1 Cloud water provides a nutritive medium for microbial growth

In order to investigate the capacity of cloud water to act as a nutritive medium for cells, a bulk cloud water sample was incubated a few hours after sampling without any supplementation in nutritive material. The evolution of the concentration in Adenosine Triphosphate (ATP), a key molecule of life, in this cloud sample is shown in Fig. 1, and the total bacteria counted at the beginning of the incubation and after 97 h at 17°C are also plotted. We observe that after a lag time of about 45 h during which ATP concentration is rather constant, activity raises. After 90 h, the ATP concentration does reach about 7 times the value that was measured at the beginning. Furthermore, during that time, while fungal cells concentration is not significantly changed, total bacteria number increased from $7.6\times10^4\pm1.3\times10^3$ mL$^{-1}$ to $1.1\times10^6\pm4.6\times10^3$ mL$^{-1}$. Such an observation suggests that bacteria find nutrients to multiply in the liquid phase of clouds, and confirms the hypothesis made by Fuzzi et al. (1997). In parallel, organic acids measurements performed by ionic chromatography on the sample we used for this experiment show the presence of potential substrates. For instance, concentrations in formate and acetate were about 4 $\mu$M, and many other organic compounds like succinate, malonate, oxalate and formaldehyde were detected (Parazols et al., unpublished data). The lag time could be attributed to the time required by cells for responding to the mechanical and thermal stresses caused by both the sampling method (impaction) and the further manipulations.
3.2 Efficiencies of biodegradation of atmospheric organic compounds by isolated strains

Biodegradation capacities of a total of 60 aerobic strains previously isolated from cloud water collected at the puy de Dôme summit (1465 m a.s.l.), including bacteria and yeasts, have been tested on formate, acetate, lactate, succinate, and on a mixed solution of formaldehyde and methanol. Numbers of 30 Gram positive, 20 Gram negative and 4 unidentified bacterial strains and 6 yeasts strains have been investigated.

Results are given separately for Gram positive, Gram negative and yeasts isolates in Fig. 2, which give an overview of the potentialities of all the tested strains. In Fig. 3, biodegradation efficiencies of strains belonging to the main bacterial genera recovered from our cloud water samples (3 strains of *Arthrobacter*, 3 of *Micrococcus*, 6 of *Bacillus*, 7 of *Staphylococcus*, 5 of *Sphingomonas* and 9 of *Pseudomonas*) (see Amato et al., 2007) and yeasts are detailed for each compound. These two figures will be discussed simultaneously all along the presentation of this section. Means and medians are generally distinct, indicating that data does not follow Gaussian shaped distributions. Medians are generally higher than means, and the former will thus be preferably considered to compare results between themselves.

On the whole, highest efficiencies of degradation are observed for Gram negative bacteria (Fig. 2). It is of first interest since Gram negative bacteria such as *Pseudomonas* species are thought to be the more active cells present in clouds, as they often develop at low temperature (Amato et al., 2007). In addition, formate, acetate and formaldehyde appear to be the more actively transformed compounds, and they are also the more concentrated organic compounds found in cloud water. All these results support a potential activity of biodegradation of those compounds in cloud water.

For formate, Gram negative bacteria, Gram positive bacteria and yeasts degrade respectively, in median, 100%, 97% and 65% of the amount present at the beginning of the test (Fig. 2). Only a few strains have a poor activity toward this compound, especially *Micrococcus*, *Sphingomonas* and yeasts strains looking at the main microor-
ganisms (Fig. 3). On the contrary, all of the strains of *Arthrobacter* and *Pseudomonas* affilations completely remove formate from the incubation media within 24 h of incubation.

A large majority of strains degrade acetate very efficiently, especially among Gram negative and yeasts isolates (Fig. 2). For the main genera present in cloud water, highest activities are noticed among *Micrococcus*, *Sphingomonas* and *Pseudomonas* groups, for which percentages of biodegradation reach 100% in most of the cases (Fig. 3). On the other hand, *Staphylococcus* species regroup the less efficient strains with a median situated under the value of 40%.

For lactate, as expected, biodegradation is dependent on the isomer form considered, and is always better in the case of L-lactate (Fig. 2). Indeed, medians are ranging from 60% (*Staphylococcus* group) to 100% (*Bacillus* and *Pseudomonas* groups) for the latter, while for D-lactate it falls to less than 50% for a majority of strains, and is even close to 0% for the entire *Staphylococcus* group (Fig. 3). Only a few strains are able to degrade D-lactate, especially among those belonging to *Micrococcus* and *Bacillus* for the selected genera (Fig. 3). For some *Staphylococcus* and yeasts strains, an increase of concentration of D-lactate is even observed (negative value of biodegradation), likely resulting from the transformation of L-lactate by a racemase.

Concerning succinate, percentages of biodegradations are very variable from one group to another (Fig. 2), often superior to 90% among Gram negative bacteria, but lower than 20% for 4 out of all the 5 *Staphylococcus* (Gram positive) species tested (Fig. 3).

Methanol and formaldehyde were studied as a mix in the incubation media due to the use of a commercial solution in which formaldehyde is stabilized by the presence of methanol. In this particular case, the rapid elimination of formaldehyde seems to be a priority for cells: almost all strains are able to transform the total amount of this compound within 24 h. A related production of methanol (resulting in a negative value of biodegradation) is consequently often observed. Figure 2 and Fig. 3 show that Gram positive bacteria highly transform both formaldehyde and methanol, while methanol of-
ten accumulates in the cases of Gram negative (especially *Pseudomonas*) and yeasts strains.

3.3 Specificities of microbial groups toward the proposed substrates

From a biochemical point of view, several metabolic behaviours toward the different compounds are encountered among the main groups isolated from cloud water (Fig. 3). First, we can argue that strains of the genus *Staphylococcus* have a great specificity for formate, being less efficient than all the other groups toward each of the other compounds. *Sphingomonas* and yeasts are poor consumers of formate and prefer acetate, L-lactate or succinate. *Micrococcus* is situated between these two extremes, degrading methanol and formaldehyde very efficiently, but not formate. *Bacillus* strains seem to have no special requirement, as well as *Arthrobacter* and *Pseudomonas*, except D-lactate for the former and methanol for the latter.

3.4 Pathways of biodegradation of carboxylic acids

The metabolic pathways involved in the biotransformation of carboxylic acids cannot be determined with certitude by solely considering the data presented here. However, it gives indications when metabolites are produced in the solution and then are detected by $^1$H NMR analysis. Table 1 reports the chemical shifts and multiplicity of signals related to the tested carboxylic acids and also to signals that can be attributed to metabolites (i.e. when they are not detected in the corresponding blank).

Formate is the shorter existing carboxylic acid, and microbial metabolism can only lead to its direct oxidation to carbon dioxide (KEGG Metabolic Pathway database) (see Fig. 4a). As a consequence, no metabolite is detected in this case.

Acetate is a link taking place in many routes of the central metabolism mainly through the complexation with Coenzyme A to enter the Krebs cycle. In addition, the uptake of acetate by cells does not require, most of the time, any carrier system as it diffuses through the cytoplasmic membrane (Kell et al., 1981). This certainly explains the high
capacity of biodegradation generally noticed whatever the microbial group considered. However, few strains have a low activity on that substrate, especially among genera *Bacillus* and *Staphylococcus* for Gram positive bacteria, and a very low value (5%) is observed for an Actinobacteria: *Frigoribacterium* sp. PDD14b-13 (DQ512796). In some cases, unidentified products were released in the incubation media containing acetate, resonating as doublets at 5.80 ppm and 7.5 ppm, or as a triplet at 1.05 ppm (Fig. 5a).

For lactate, different metabolic pathways exist depending on the isomer form L or D (Fig. 4b). A racemase is known to be involved in the transformation of an isomer form of lactate to the other (Kitahara et al., 1953), but is rarely encountered referring to the KEGG database. For both forms, lactate can enter the central microbial metabolism either by oxidation to pyruvate or reduction to lactaldehyde (it can also make a complex with Coenzyme A; not shown on the scheme). A fourth way involves only L-lactate, and consists in its decarboxylation, leading to the production of acetate. Acetate ($\delta=1.92$ ppm) and pyruvate ($\delta=2.38$ ppm) productions were actually unambiguously identified as metabolites, while the apparition of non identified triplet signals is often observed ($\delta=2.4$ ppm and 3.0 ppm) (Fig. 5b). Interestingly, as D-lactate concentration increases in the presence of the two isomers (leading to a negative value of biodegradation), two Gram negative bacterial strains likely express a lactate racemase: *Methylobacterium* sp. PDD7b-5 (DQ512770) and *Flavobacterium* sp. PDD14b-7 (DQ512791). The presence of low levels of pyruvate in cloud water was reported by Löflund and collaborators (2002), and one could see here the signature of such a biological activity.

Succinate is largely involved in the central metabolism (Krebs cycle) and represents a metabolic link between succinyl-CoA and fumarate (Fig. 4c). Its oxidation to fumarate is directly linked to the production of ATP, as it takes place in the oxidative phosphorylation. We often observe the production of a compound resonating as a singlet at 6.5 ppm, which we assigned to the signal of the CH group in fumarate (HOOC-HC=CH-COOH) (Fig. 5c). A release of acetate is also observed in some cases, as well as an
unidentified product resonating as a triplet centred at 3.0 ppm. The latter can also corresponds to one of the two triplets observed under incubation with lactate ($\delta = 2.4$ ppm and 3.0 ppm), the other being masked by the signal of the succinate itself. Other non assigned singlets also appear for some strains incubated in the presence of succinate.

3.5 Metabolism of methanol and formaldehyde

The values of biodegradation determined for methanol show a large dispersion, ranging from about –80% (production of methanol) to 100% (Fig. 2). The concentration of methanol measured in our analysis results from the equilibrium between on one hand its degradation to formaldehyde, and on one other hand its production from formaldehyde (see Fig. 3a). All microbial groups are very efficient to degrade formaldehyde, and this compound consequently does not accumulate when methanol is oxidized. Considering the known high toxicity of formaldehyde, the priority for the cells is likely to eliminate it early, and in many cases methanol concentration is thus increasing within the 24 h of the test.

In order to identify more clearly and with certainty the metabolites produced in relation to formaldehyde, we incubated one selected strain in the presence of pure (meaning single) $^{13}$C labelled formaldehyde. This strain was arbitrary chosen amongst bacteria for its interesting behaviour toward the mixed solution of formaldehyde /methanol. It was identified as an Actinobacterium: Frigoribacterium sp. PDD14b-13 (DQ512796).

Four fates of formaldehyde are known to be possible through microbial metabolic pathways (Fig. 3a): (i) its assimilation by the serine and/or the ribulose monophosphate pathways (the latter involves a decarboxylation); (ii) its reduction to methanol; (iii) its oxidation to formate; and (iv) its reaction with methanol, forming methylformate (HCOOCH$_3$) (Mason and Sanders, 1989; Murdanoto et al., 1997; Delort, 2006). The second and third ways involve an oxydoreductase and/or a dismutase (Kato et al., 1984), the latter simultaneously producing formate and methanol from two molecules of formaldehyde. In addition, several known formaldehyde dehydrogenases are only dedicated to formaldehyde reduction (Vorholt, 2002), and a methanol dehydrogenase
also exists, catalyzing the production of formaldehyde. Analyses of the related supernatants by $^{13}$C NMR clearly show that this strain of *Frigoribacterium* highly transforms formaldehyde to formate and methanol (Fig. 6). After less than 4 h, no formaldehyde remains in the solution that contained approximately 2 mM at the beginning. Then, formate is oxidized to CO$_2$, while methanol is still not transformed after 24 h. Obviously, $^1$H NMR spectrum of the same supernatants also show the presence of $^{13}$C formate and $^{13}$C methanol, the doublets indicating that these compounds are actually labelled with $^{13}$C originating from formaldehyde. In addition, other compounds are released, corresponding to signals at 1.45 ppm, 1.95 ppm, 2.4 ppm and 2.15 ppm, resonating as singlets. Such signals can correspond either to non labelled metabolites due to a decarboxylation related to their assimilation (loss of the $^{13}$C to $^{13}$CO$_2$) or to metabolites produced from endogenous compounds of cells.

In the atmosphere, formaldehyde is strongly linked to free radicals chemistry (Satsumabayashi et al., 1995; Riedel et al., 1999; Kawamura et al., 2005), and is so of first interest. Monod et al. (2000) studied photochemistry of methanol in aqueous phase and showed that free radicals produced by solar light also oxidize methanol to formaldehyde and formate. The results obtained here show that strains found in clouds can be responsible of the same transformations than those induced by photochemistry.

### 4 Conclusions

In this study we investigated the interactions between micro-organisms and the chemical environment provided by cloud water. First we observed that, as it was discussed for several years, cloud water solution can provide a niche for the multiplication of bacteria, obviously supported by the organic compounds it contains. As a consequence, the uptake of chemical compounds by micro-organisms could have an effect on the whole composition of cloud water, assumed to be of first importance in atmospheric chemistry. The biodegradation tests of various single organic compounds present in cloud water performed on 60 microbial strains (bacteria and yeasts) previously isolated from cloud
water samples showed a high activity on acetate, formate, L-lactate and formaldehyde. These are also the main organic compounds found in cloud water. Biodegradation of D-lactate appears to be relatively low. Many genera often encountered in cloud water, like *Pseudomonas* or *Bacillus*, highly degrade all of the compounds tested. On the contrary, *Staphylococcus* species are very specialized toward formate degradation. For succinate, results are highly variable depending on the microbial groups.

Several metabolites resulting from the transformation of the compounds investigated were detected, like pyruvate from lactate oxidation, or fumarate from succinate. These compounds were actually detected in clouds, and their presence could thus be partially attributed to such a microbial activity. More than being simply of biochemical interest, these results clearly show that cells can be a sink, but also a source of organic compounds for cloud water.

Metabolisms of methanol and formaldehyde are strongly linked, and incubations in the presence of $^{13}$C labelled formaldehyde bring the proof that both oxidation and reduction of formaldehyde can be microbiologically catalysed by strains found in cloud water. As such reactions also involve photochemistry, microbiological and photochemical processes can act conversely or simultaneously on the chemistry of cloud water. Consequently, the concentration of a given compound can be negatively as well as positively influenced by biology. Thus actual models of atmospheric chemistry could under- or overestimate the weight of some reactions by only considering the reactivity due to free radicals. The relatively poor knowledge about the respective contribution of microbiology versus free radical does not allow any conclusion about an actual participation of micro-organisms in atmospheric chemistry, but experimental results in favour of such a hypothesis are constantly improving.

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References


Kawamura, K., Steinberg, S., Ng, L., and Kaplan, I. R.: Wet deposition of low molecular weight


Table 1. $^1$H NMR signals of the tested organic acids and metabolites detected.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift (ppm)</th>
<th>Metabolite(s) detected</th>
</tr>
</thead>
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<tr>
<td>Formate</td>
<td>H-COO$^-$</td>
<td>None</td>
</tr>
<tr>
<td>Acetate</td>
<td>$\text{H}_3\text{C-COO}^-$</td>
<td>Triplet at 1.05 ppm not attributed Doublets at 5.80 ppm and 7.55 ppm not attributed</td>
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<tr>
<td>Lactate</td>
<td>$\text{H}_3\text{C-CHOH-COO}^-$</td>
<td>Acetate (1.92 ppm, singlet) Pyruvate (2.38 ppm, singlet) Triplet at 2.4 and 3.0 ppm not attributed</td>
</tr>
<tr>
<td>Succinate</td>
<td>$\text{OOC-(CH}_2)_2\text{-COO}^-$</td>
<td>Acetate (1.92 ppm, singlet) Fumarate (6.50 ppm, singlet) Triplet at 3.00 ppm not attributed Singlets at 1.35 ppm, 1.50 ppm and 5.45 ppm not attributed</td>
</tr>
</tbody>
</table>
**Fig. 1.** Timedependence of the concentration of adenosine tri-phosphate (ATP) in a bulk cloud water sample stored at 17°C under agitation (200 rpm) and closed against exogenous contamination. Total cells were counted by epifluorescence microscopy at the beginning and at the end of the experiment. Fungal cells (not plotted) were respectively $2.7 \pm 0.6 \times 10^3 \text{mL}^{-1}$ and $1.5 \pm 0.6 \times 10^3 \text{mL}^{-1}$. Errors bars were obtained from triplicate measurements of ATP concentration but are masked by symbols.
Fig. 2. Percentages of biodegradation for each compound by Gram positive and Gram negative bacteria and yeasts after 24 h of incubation. Boxes represent medians (—) and 25–75 percentiles; □ means; × minima and maxima. The number of strains considered in each case is indicated. Median is not precised when n<5.
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in each case is indicated. Median is not precise when n<5.

Fig. 3. Percentages of biodegradation for each compound by main genus of bacteria and total yeasts found in cloud water after 24 h of incubation (Arthrobacter, Micrococcus, Bacillus, Staphylococcus, Sphingomonas and Pseudomonas and yeasts). Boxes represent medians (—), 25–75 percentiles; ✷ means; × minima and maxima. The number of strains considered (n = 3–9) and 25–75 percentiles: medians × minima and maxima. The number of strains considered — and 

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bacteria (n)</th>
<th>Yeasts (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formate</td>
<td>Arthrobacter 3</td>
<td>Yeasts 6</td>
</tr>
<tr>
<td>Acetate</td>
<td>Arthrobacter 3</td>
<td>Yeasts 6</td>
</tr>
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<td>L-Lactate</td>
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<td>D-Lactate</td>
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<td>Yeasts 6</td>
</tr>
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<td>Succinate</td>
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</tr>
<tr>
<td>Methanol</td>
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<td>Yeasts 6</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Arthrobacter 2</td>
<td>Yeasts 6</td>
</tr>
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</table>
Fig. 4. Schematic representation of known metabolic pathways involving methanol, formaldehyde and formate (a), lactate (b) and succinate (c).
Chemical shift (ppm)

Fig. 5. Examples of $^1$H NMR spectra showing signals appearing during incubation with acetate (a), lactate (b) and succinate (c). Spectra presented on (a) were obtained with strains of Bacillus sp. (upper trace)) and Curtobacterium flaccumfaciens (lower trace); on (b) Pseudomonas viridiflava and on (c) Curtobacterium flaccumfaciens. The large signal around 5 ppm corresponds to the peak of water.
Fig. 6. (a) ${}^{13}$C NMR spectra showing the transformation of ${}^{13}$C formaldehyde present at the beginning into ${}^{13}$C methanol and ${}^{13}$C formate during the time of incubation (example of the strain *Frigoribacterium* sp. PDD14b-13 (DQ512796) incubated at 17°C. (b) Corresponding ${}^1$H NMR spectra, with doublets related to ${}^{13}$C labelled compounds. Singlet signals are related to non labelled compounds.