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To cite this version:

Pierre Amato, Florence Demeer, Amina Melaouhi, Stéphane Fontanella, Anne-Sophie Martin-Biesse, et al.. A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms. Atmospheric Chemistry and Physics, 2007, 7 (15), pp.4169. 10.5194/acp-7-4159-2007 . hal-00328519

HAL Id: hal-00328519

https://hal.science/hal-00328519v1

Submitted on 18 Jun 2008

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Atmos. Chem. Phys., 7, 4159–4169, 2007
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Atmospheric Chemistry and Physics

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

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Received: 19 March 2007 – Published in Atmos. Chem. Phys. Discuss.: 18 April 2007
Revised: 18 July 2007 – Accepted: 7 August 2007 – Published: 9 August 2007

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 examined for their ability to degrade some of the main atmospheric carboxylic compounds: formate, acetate, lactate, succinate, as well as 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.

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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 microorganisms in clouds was estimated to reach 1 to 10 Tg C per year (Sattler et al., 2001). During previous investigations, we isolated a large variety of bacterial and fungal microorganisms from cloud water samples in which ATP concentration implied microbial activity (Amato et al., 2007b). 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_1 compounds, the transformation of methanol to formaldehyde, formate and finally to CO_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 address this deficiency, we present in this article results obtained from a large study, involving 60 microbial 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., 2007b). 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 introductory 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 Amato 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 throughout the experiment. ATP concentration was measured by bioluminescence (luciferin/luciferase) for 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 measurements were made with a bioluminometer Biocounter M2500 (Lumac). In addition, total cell counts have been carried out by epifluorescence microscopy at the beginning and at the end of the incubation, in triplicates. Volumes of 7.5 mL were fixed with equal volumes of prefiltered 4–5% formaldehyde, 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 ×40 for fungal cells, then ×100 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 defined volume of culture with addition of about 20 mM of sodium formate (Aldrich), lactic acid (mix of isomers L and D, approximately 70:30) (Touzard and 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 methanol) in 0.1 M phosphate buffer at pH 7.0. The volume of culture used was adjusted based on its $OD_{580\,nm}$ to keep constant the ratio between cell density and concentration of the compound to degrade. For a given strain, all tests were performed using cells originating from a same culture flask, which was distributed between the tests solutions. 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 also systematically made. Incubations were carried out in 100 mL Erlenmeyer's flasks containing a final volume of 25 mL, under agitation (200 rpm), at 17°C or 27°C depending on the optimal conditions for growth of the strain. Samples of about 1 mL were taken at the beginning and after 24 h of incubation, centrifuged (12 000 g, 3 min) and 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 μl of the sample with 50 μl of sodium tetra deuteriated trimethylsilyl propionate ($TSPd_4$, Eurisotop) in solution in D_2O. The latter was used for locking and shimming, while $TSPd_4$ constituted a reference for chemical shifts (0 ppm) and quantification. 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: $[m]=(9*Ao*[TSPd_4])/(b*A_{ref})$, where $[m]$ is the concentration of the compound to quantify, Ao is the area

of m resonance, A_{ref} is the area of $TSPd_4$ resonance, and 9 and b are respectively the numbers of protons of $TSPd_4$, 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, and therefore is masked by the signal of water. A sensitive automatic analyser was used (Aerolaser AL4021). The principle is based on the reaction of formaldehyde with acetylacetone and ammoniac, resulting in the formation of the detected fluorochrome complex ($\lambda_{excitation}$=400 nm; $\lambda_{emission}$=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. Incubations 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 as 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 collected in January 2005 was incubated a few hours after sampling without any supplementation in nutritive material. During sampling time, temperature averaged −3.2°C, pH was 6.8. This value of pH attests that this cloud event was not under anthropogenic influence. The evolution of the concentration of Adenosine Triphosphate (ATP), a key molecule for life, in this cloud sample incubated at 17°C is shown in Fig. 1, and the total bacteria counted at the beginning of the incubation and after 97 h are also plotted. We observe that after a lag time of about 45 h during which ATP concentration is rather constant, activity raises. This 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. After 90 h, the concentration of ATP reaches

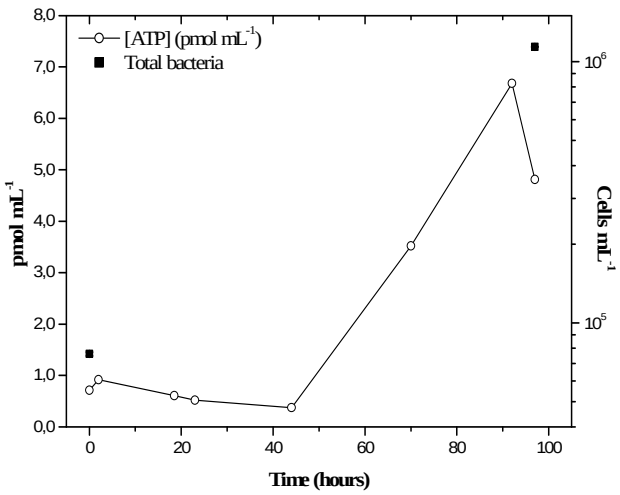

Fig. 1. Time-dependence of the concentration of adenosine triphosphate (ATP) in a bulk cloud water sample stored at 17°C under agitation (200 rpm) and closed against exogenous contamination. Total cells were counted by epifluorescence microscopy at the beginning and at the end of the experiment. Fungal cells (not plotted) were respectively $2.7\pm0.6\times10^3$ mL^{-1} and $1.5\pm0.6\times10^3$ mL^{-1}. Errors bars were obtained from triplicate measurements of ATP concentration but are masked by symbols.

about 7 times the value measured at the beginning. Furthermore, during that time, while fungal cells concentration is not significantly changed, total bacteria number is 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 clearly suggests that bacteria find nutrients to multiply in the liquid phase of clouds, and confirms the hypothesis made by Fuzzi et al. (1997). Chemical analysis of the sample (before incubation) showed that total organic carbon (TOC) was 2.8 mg L^{-1}, with for instance presence of compounds like formate and acetate (4 μM). Many other organic compounds were also measured or at least detected (succinate, malonate, oxalate, formaldehyde...) (Parazols et al., unpublished data). Ammonium NH_4^+ and nitrate NO_3^- were respectively about 50 μM (900 μg L^{-1}) and 25 μM (1550 μg L^{-1}) and elements such as P, S, Fe, Mg, Na, K were present. Regarding these data and assuming that a C/N/P ratio of 100/10/1 is required for growth, carbon is the nutritive limiting factor for cell multiplication in clouds.

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

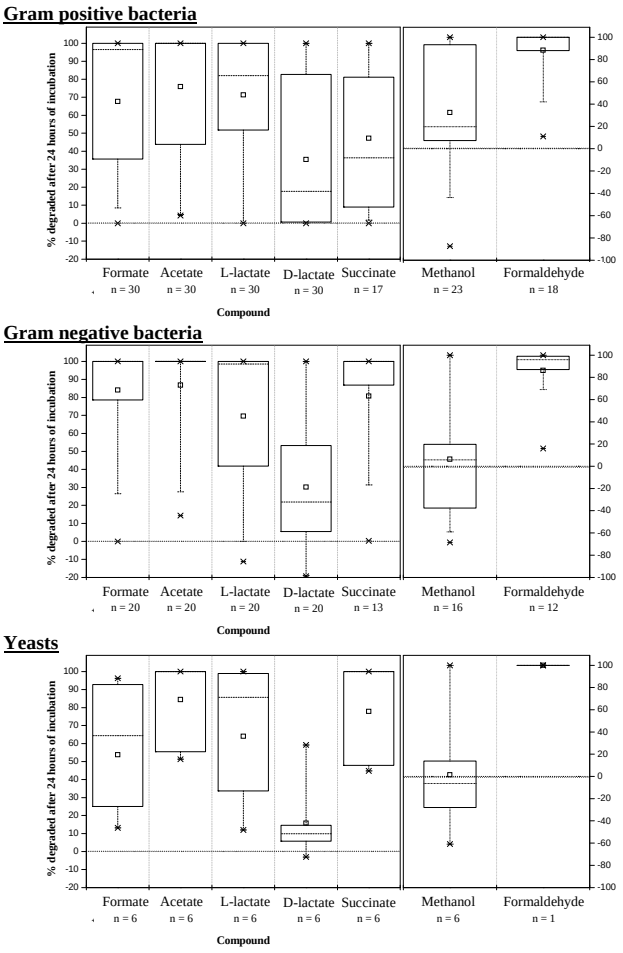

Fig. 2. Percentages of biodegradation for each compound by Gram positive and Gram negative bacteria and yeasts after 24 h of incubation. Boxes represent medians (- - -) and 25–75 percentiles; □ means; × minima and maxima. The number of strains considered in each case is indicated. Median is not precised when $n<5$.

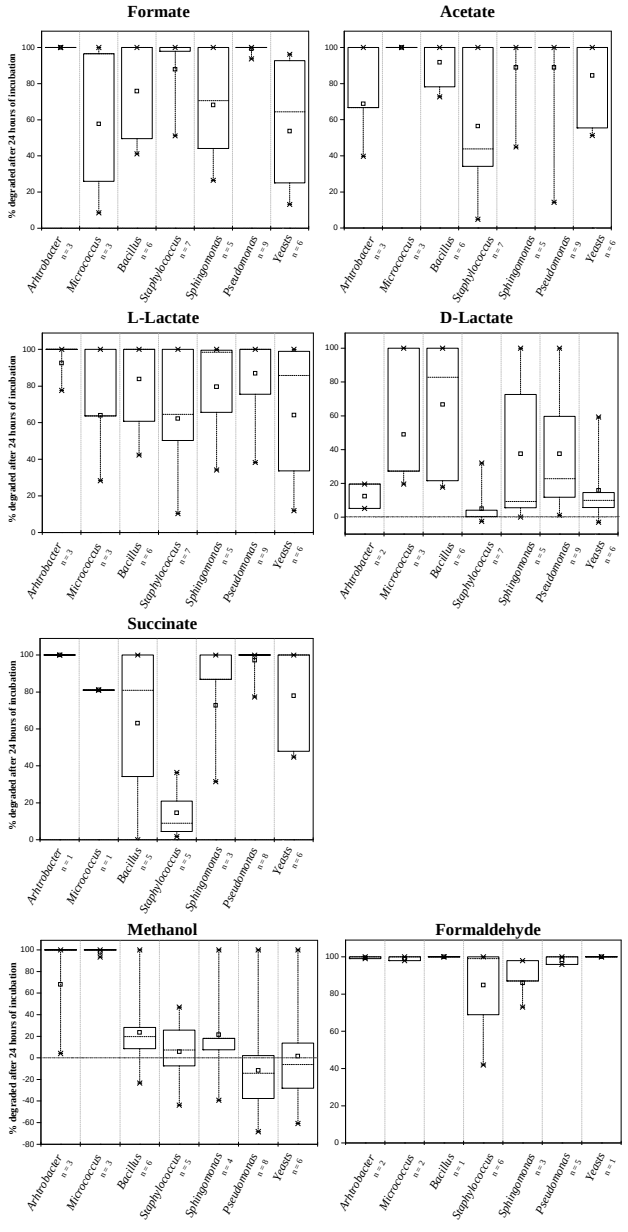

Fig. 3. Percentages of biodegradation for each compound by main genera of bacteria and total yeasts found in cloud water, after 24 h of incubation (*Arthrobacter*, *Micrococcus*, *Bacillus*, *Staphylococcus*, *Sphingomonas* and *Pseudomonas* and yeasts). Boxes represent medians (- - -) and 25–75 percentiles; □ means; × minima and maxima. The number of strains considered in each case is indicated. Median is not precised when $n<5$.

investigated. NMR was used as analytical method as this allows both detection and quantification of organic compounds, rapidly and without any presumption.

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

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., 2007a). In addition, formate, acetate and formaldehyde

Table 1. Carboxylic acids tested, ^{1}H NMR chemical shifts of the groups in bold (respect to $TSPd_4$, at pH 7.0) and related metabolites detected in the incubation media.

Compound		Chemical shift (ppm)	Metabolite(s) detected
Formate	**H**-COO$^-$	8.46, singlet	None
Acetate	**H**$_3$C-COO$^-$	1.92, singlet	Triplet at 1.05 ppm not attributed Doublets at 5.80 ppm and 7.55 ppm not attributed
Lactate	**H**$_3$C-CHOH-COO$^-$	1.45, doublet (L isomer) 1.33, doublet (D isomer)	Acetate (1.92 ppm, singlet) Pyruvate (2.38 ppm, singlet) Triplets at 2.4 and 3.0 ppm not attributed
Succinate	$^-$OOC-(C**H**$_2$)$_2$-COO$^-$	2.41, singlet	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

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 microorganisms (Fig. 3). On the contrary, all of the strains of *Arthrobacter* and *Pseudomonas* affiliations completely remove formate from the incubation media within 24 h.

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 it reaches less than 50% for a large majority of strains for D-lactate, 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* (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 biodegradation are very variable from one group to another (Fig. 2), often greater than 90% among Gram negative bacteria, but lower than 20% for 4 out of the 5 *Staphylococcus* strains (Gram positive) (Fig. 3).

Methanol and formaldehyde were studied mixed in the incubation media since a commercial solution in which formaldehyde is stabilized by the presence of methanol was used. 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. Figures 2 and 3 show that Gram positive bacteria highly transform both formaldehyde and methanol, while methanol often 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

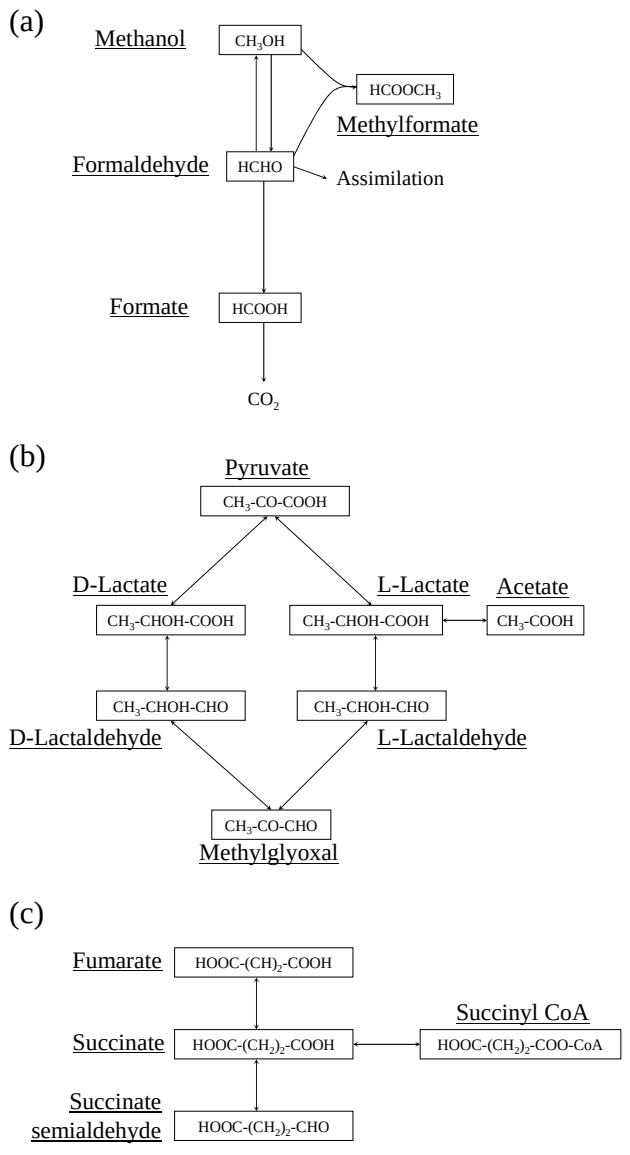

Fig. 4. Schematic representation of known metabolic pathways involving methanol, formaldehyde and formate **(a)**, lactate **(b)** and succinate **(c)**.

indications when metabolites are released in the solution and are consequently detected by ^{1}H NMR analysis. Table 1 reports the chemical shifts and multiplicity of signals related to the tested carboxylic acids, and of signals that can be attributed to metabolites (i.e. when they are not detected in the corresponding blank).

Formate is the smaller 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 for this compound.

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 present a low activity toward this 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 are 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 link 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 (δ=1.92 ppm) and pyruvate (δ=2.38 ppm) productions were actually unambiguously identified, while the apparition of non identified triplet signals is often observed (δ=2.4 ppm and 3.0 ppm) (Fig. 5b). Interestingly, as D-lactate concentration increases when both isomers are present (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). Low levels of pyruvate in cloud water have been 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. A compound resonating as a singlet at 6.5 ppm is produced in many cases. This signal likely corresponds to the CH group in fumarate (HOOC-HC=CH-COOH) (Fig. 5c). A release of acetate is also observed, as well as an unidentified product resonating as a triplet centred at 3.0 ppm. The latter may also be related to one of the two triplets detected by incubation with lactate (δ=2.4 ppm and 3.0 ppm), the other being masked by the signal of succinate itself. Other non assigned singlets are also detected following the biodegradation of succinate.

3.5 Metabolism of methanol and formaldehyde

Values of biodegradation determined for methanol are highly variable, ranging from about −80% (production of methanol) to 100% (Fig. 2). The concentration of methanol measured

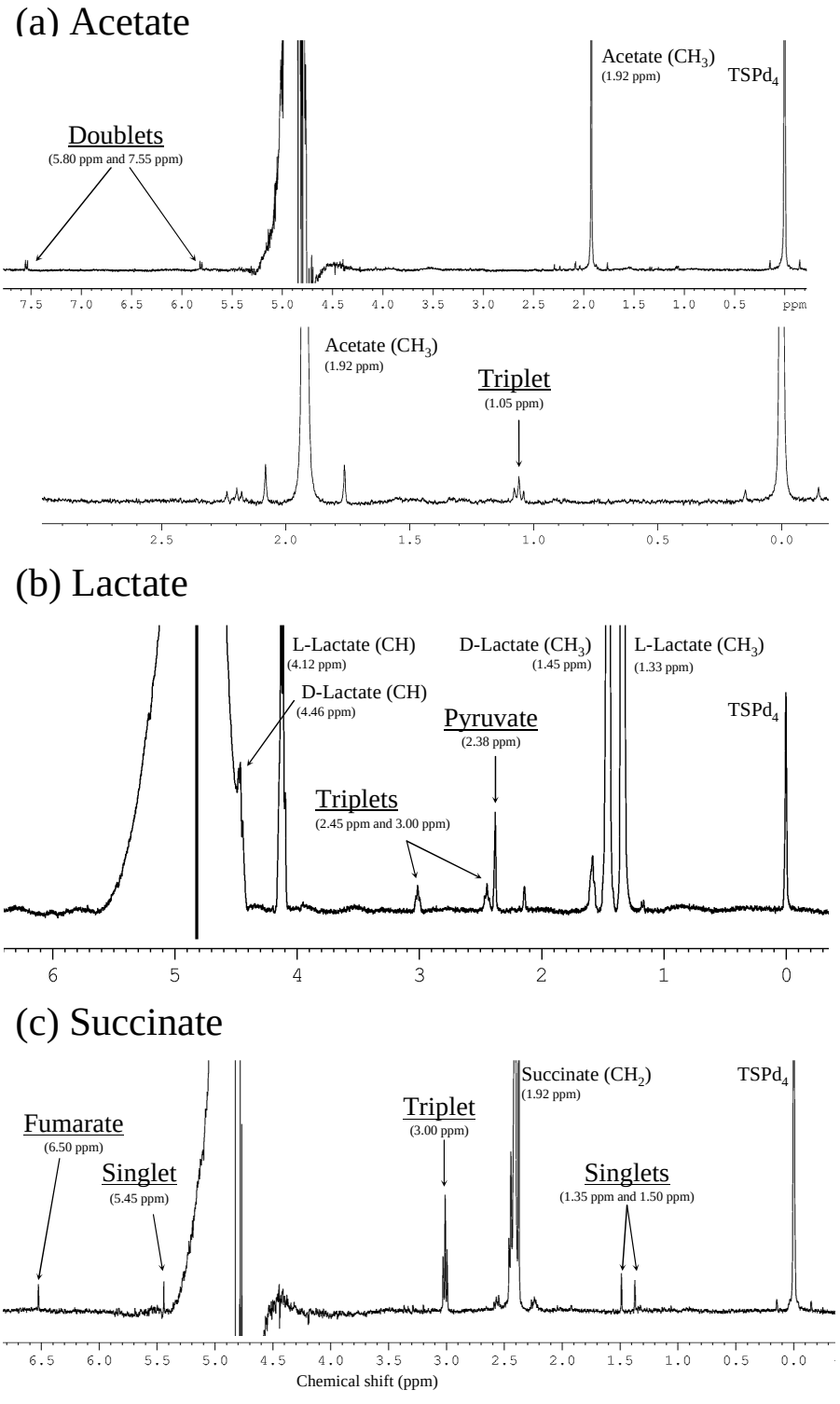

Fig. 5. Examples of ^{1}H NMR spectra showing signals appearing during incubation with acetate **(a)**, lactate **(b)** and succinate **(c)**. Spectra presented on (a) were obtained with strains of *Bacillus* sp. (upper trace)) and *Curtobacterium flaccumfaciens* (lower trace); on (b) *Pseudomonas viridiflava* and on (c) *Curtobacterium flaccumfaciens*. The large signal around 5 ppm corresponds to the peak of water.

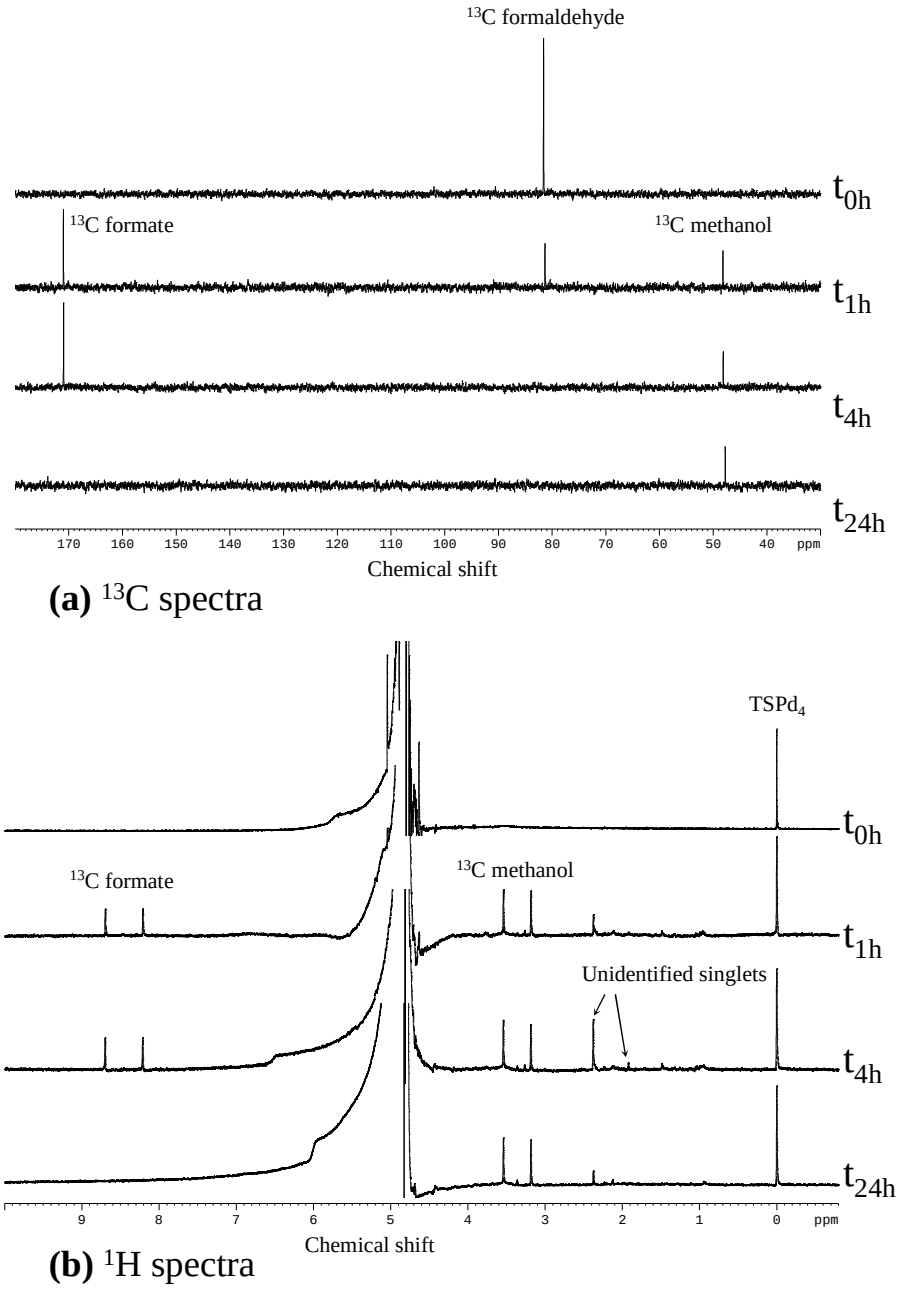

Fig. 6. (a) ^{13}C NMR spectra showing the transformation of ^{13}C formaldehyde present at the beginning into ^{13}C methanol and ^{13}C formate during the time of incubation (example of the strain *Frigoribacterium* sp. PDD14b-13 (DQ512796) incubated at 17°C. **(b)** Corresponding ^{1}H NMR spectra, with doublets related to ^{13}C labelled compounds. Singlet signals are related to non labelled compounds.

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 degrade formaldehyde very efficiently, and the latter consequently does not accumulate when methanol is oxidized. Considering the known high toxicity of formaldehyde, the priority for the cells is likely to reduce its concentration, and in many cases methanol concentration is thus increasing within the 24 h of the test.

In order to unequivocally identify the metabolites produced from 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 catalyzes 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 containing approximately 2 mM at the beginning. Then, formate is oxidized to CO_2, while methanol is still not transformed after 24 h. ^{1}H NMR spectrum of the same supernatants also exhibit the presence of ^{13}C formate and ^{13}C methanol, the doublet-shaped signals indicating that 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 possibly correspond either to non labelled metabolites due to a decarboxylation following their assimilation (loss of the ^{13}C to $^{13}CO_2$) or to metabolites produced from endogenous compounds. In the atmosphere, formaldehyde is strongly linked to free radical 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.

4 Conclusions

In this study we investigated 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 supported by the organic compounds it contains. As a consequence, the uptake of chemical compounds by micro-organisms could have an effect on the composition of cloud water, assumed to be of primary importance in atmospheric chemistry. 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 show 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. Various trends are observed, partially depending on the genus considered. For instance, *Staphylococcus* species are specialized toward C_1 compounds degradation, and such trends have to be kept in mind for the further investigations.

Several metabolites were detected, like pyruvate from lactate oxidation, or fumarate from succinate. These compounds were actually measured in cloud samples, and their presence could thus be partially attributed to such a microbial activity. More than being simply of biochemical interest, this clearly shows that cells can represent a sink, but also a source of organic compounds in 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 microbes found in cloud water. Such reactions are also known to be induced by light. Hence, 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 considering only the reactivity linked to photochemistry.

Though the chosen experimental conditions do not match those encountered in clouds (these environments consist in a complex solution distributed in micron sized droplets, and some physico-chemical properties can be limiting for metabolism: low temperature and pH, high UV light and oxidative capacity), our investigations demonstrated that microorganisms present in clouds have an enzymatic pool that could potentially significantly affect atmospheric organic chemistry.

The large overview presented here shows that further research is needed to explain fully microbiological contribution to cloud chemistry since a real ability is depicted. Some hints are given and characteristics of cloud water should now be considered by experimental conditions, with the goal to approach or even mimic a real cloud. The relatively short lifetime of a cloud droplet also has to be taken in account. Anthropogenic pollution is known to prolong the lifetime of clouds, then microbial effects should be greater under these circumstances. But as a first step, we recommend having a special emphasis on the genus *Pseudomonas*, among which the most rapidly metabolizing bacteria were observed.

In order to figure out what is the influence of each limiting factor on the efficiency of biodegradation, each of them has to be added in the experimental conditions at once. Temperature is certainly one of the most important of these factors, and the next step is thus to determine metabolic constants under low temperature.

Acknowledgements. The research was funded by the French LEFE-CHAT from the CNRS and the ORE-BEAM program of the French Ministry of Research. P. Amato is also the recipient of a scholarship from the French Ministry of Research. A. Melaouhi is under an AUF fellowship. E. Cano is greatly acknowledged for her technical help.

Edited by: J. Williams

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