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BACTERIAL UTILIZATION OF FORMIC AND ACETIC ACID IN RAINWATER

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Abstract—Rain samples were collected aseptically, during 1983 and 1984, in Charlottesville, Virginia to determine the ability of bacteria in precipitation to utilize formate and acetate. The total number of bacteria, as counted by Acridine Orange Direct Counts, was one to two orders of magnitude greater from April to September (10^5 cells ml^{-1}) than during the rest of the year (10^3–10^4 cells ml^{-1}). Formate and acetate concentrations ranged between 6–23 and 3–9 μM, respectively and were higher from June to September. Heterotrophic uptake on the day of collection was not different from the controls, but after incubation at room temperature for a minimum of three days, the turnover rate constants were 0.14 and 0.17 h^{-1} for formate and acetate, respectively. Total bacterial counts increased an order of magnitude during that interval. These turnover rate constants were used to calculate losses of 44 and 24 μmol ℓ^{-1} day^{-1} of formic and acetic acid, respectively. Turnover times were 1.5 and 34 days for formate and acetate, respectively. This study demonstrated that there are viable microorganisms in the atmosphere capable of utilizing formate and acetate for growth.

Key word index: Acetic acid, formic acid, acid rain, acid deposition, atmosphere, bacteria, precipitation.

INTRODUCTION

Acid deposition has become an issue of global concern in the past few decades. Once considered a local problem, it is now recognized that long-distance transport of atmospheric pollutants can affect the chemistry of precipitation thousands of km away from the pollution source (Cowling, 1980; Swedish Ministry of Agriculture, 1982; National Research Council, 1986). Although the most commonly recognized sources of acidity in precipitation are sulfuric and nitric acids which originate from both natural and anthropogenic emissions (Galloway *et al.*, 1976; Cowling and Linthurst, 1981; Charlson and Rodhe, 1982; Chadwick, 1983), weak acids (i.e. organic acids such as formic and acetic acid) also contribute acidity to precipitation. Measurements and indirect estimates indicate that organic acids may contribute between 16 and 35% of the free acidity to precipitation in the United States, especially in areas unimpacted by acid deposition (Keene and Galloway, 1984a). Organic acids are major chemical constituents of precipitation from remote regions of the world, contributing 66 and 41% of the volume-weighted free acidity in San Carlos, Venezuela and Katherine, Australia, respectively (Galloway *et al.*, 1982; Keene *et al.*, 1983; Keene and Galloway, 1984b). Possible sources of organic acids (specifically formic and acetic acid) in the atmosphere are volatilization of plant material, photochemical oxidation of precursor hydrocarbons and aqueous phase oxidation of formaldehyde to formic acid (Dawson *et al.*, 1980; Graedel and Weschler, 1981; Chameides and Davis, 1983; Jacob, 1986; Keene and Galloway, 1986).

Formic and acetic acids disappeared from unpreserved precipitation samples stored for 60 days even when refrigerated (Keene *et al.*, 1983). When the samples were fixed with a small amount of chloroform, loss of the acids did not occur. This 'disappearance' is strongly suggestive of microbial activity in those precipitation samples. The hypothesis that microorganisms capable of utilizing organic acids exist in precipitation is supported in several ways. First, bacteria can be recovered from aerosols and precipitation (Visser, 1964; Mandrioli *et al.*, 1973; Bovallius *et al.*, 1978; Mancinelli and Shulls, 1978; Jones and Cookson, 1983), and there is ample evidence for microbiological activity in the atmosphere (Parker, 1968; Visser, 1964; Dimmick *et al.*, 1975; Straat *et al.*, 1977; Dimmick *et al.*, 1979a, b). Furthermore, formic and acetic acids are common chemical constituents in precipitation (Galloway *et al.*, 1982; Likens *et al.*, 1983; Galloway and Gaudry, 1984; Keene and Galloway, 1984a, b). These facts, coupled with the observation that microbes rarely fail to exploit an available energy source, lead to speculation as to whether microorganisms actively utilize organic acids in clouds, the dry atmosphere or both.

The present study was undertaken to determine the abundance of bacteria in central Virginia precipitation, to determine if the bacteria were capable of using formic and acetic acids, and if so, to quantify the rates of formic and acetic acid utilization. The emphasis was on the acitivity in precipitation, therefore this study focused on the bacteria present in precipitation since

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filamentous fungi would likely be present as spores and would not be expected to contribute appreciably to *in situ* metabolism. In order to meet these objectives, precipitation samples were collected aseptically in Charlottesville, Virginia during 1983 and 1984. Samples were analyzed for total number of bacterial cells, for heterotrophic activity and for concentrations of formic and acetic acids.

Bacterial cells were always present in freshly collected precipitation and the abundance was substantially higher during the warmer months of the year. Microbiological uptake of the acids could not be detected immediately after collection, but was quantifiable after the samples had incubated for four days. Turnover times obtained from kinetics experiments were consistent with published rates of acetate and formate loss from unpreserved precipitation samples. The results lead to the conclusion that there are viable microorganisms in precipitation that are capable of utilizing formate and acetate for growth, and suggest the potential for atmospheric activity of such microbes as well.

METHODS

Collection site

The study area was on the southwest side of Charlottesville, Virginia, at Clark Hall, University of Virginia. Samples were collected on a fourth-floor balcony approximately 12 m above the ground and roughly 150 m from a moderately traveled road (Jefferson Park Avenue). The funnels were placed on a table approximately 1 m above the floor, and at least 3 m away from the building wall in order to prevent splash contamination from the floor and roof, respectively. Thirty-seven separate precipitation events were sampled between 20 November 1983 and 5 December 1984.

Sample collection

Precipitation samples were collected in autoclaved vessels that consisted of 25.4-cm i.d. polypropylene funnels (Belart) supported by either 500 or 250 ml Erlenmeyer flasks. The funnels and flasks were covered with Al foil and sealed with tape prior to autoclaving. The foil was removed immediately before sample collection began and replaced and resealed immediately upon the end of collection. The sterility of the apparatus was tested by pouring autoclaved deionized water (DIW) through the apparatus and treating it as a precipitation sample. Neither the measured activity nor the concentration of cells in the water were significantly different from sterile DIW that had not been passed through the collectors, indicating no contamination from the collection apparatus.

Within 24 h of the end of a precipitation event, two 10 ml aliquots for microbial counts were fixed with formaldehyde (final concentration 2%). An aliquot of approximately 100 ml for chemical analyses was fixed with 0.5 ml chloroform (Galloway *et al.*, 1982) and stored at 4°C in a 250 ml polypropylene bottle. The remainder of the sample was used for biological uptake experiments and was left in the Erlenmeyer flask(s), covered with Al foil, until the experiments began. Aseptic technique was used during the transfer of all aliquots to prevent contamination from the air.

Chemical analyses

The concentration of total (dissociated plus undissociated) formate and acetate were measured by ion exclusion chromatography (ICE) (Keene *et al.*, 1983), on a Dionex 14 ion chromatograph. Samples were eluted with 0.00125 N HCl and all were amended with concentrated HCl to make the final concentration of HCl in the sample equal to that of the eluent. The eluent flow rate was 46 ml h^{-1} with a sample loop volume of 500 μl. Due to gain drifts during operation of the IC, mid-range standard solutions were injected after every five or six samples and gain shifts were linearly incremented through samples between each set of standards.

Microbiological analyses

Total number of cells. Total bacterial cell numbers were counted using the Acridine Orange Direct Count (AODC) staining method (Daley and Hobbie, 1975; Hobbie *et al.*, 1977; Bowden, 1977). Because of low numbers of cells in the precipitation, 10 ml of the sample was filtered for staining and counting.

Biological uptake. The relative rates of formate and acetate uptake (assimilation and mineralization) were measured using ^{14}C-formate and 2-^{14}C-acetate (New England Nuclear, formate specific activity 51.5 mCi mMol $^{-1}$; acetate specific activity 53 mCi mMol $^{-1}$) according to the methods of Wright and Burnison (1979). The 10 ml precipitation samples were incubated with either 0.45 μCi ^{14}C-formate (0.87 μM) or 0.075 μCi ^{14}C-acetate (0.14 μM) at room temperature for 4 h. After incubation, the uptake flasks were disassembled and the three fractions (^{14}C-CO_2, ^{14}C-cell assimilated and ^{14}C-unreacted substrate) were determined by liquid scintillation counting methods.

Rates of biological uptake. The amount of ^{14}C uptake (respired plus assimilated) was divided by the total amount of label added and by the length of the incubation to yield a turnover rate constant, expressed as inverse time (h^{-1}). The turnover rate constants were expressed as the average of triplicate samples when there was enough volume, and duplicates otherwise. The turnover rate constant multiplied by the *in situ* concentration of substrate yields a turnover rate, expressed as μM substrate time^{-1}, which represents the total amount of substrate the microbes assimilate and respire per unit time.

The level of activity in the freshly collected precipitation samples was below detection. Two alternative methods were used in order to measure uptake rates. In the first, the sample was allowed to incubate at room

temperature for longer than two days, thereby allowing cell numbers to increase. Heterotrophic uptake was then measured as described above. In the second, Michaelis–Menten uptake kinetics were assumed as a means of quantifying heterotrophic acitivity in freshly collected precipitation samples. T_t, the turnover time, or the amount of time required for the microorganisms to utilize the natural substrate present in the sample was calculated by using the Wright and Hobbie modification of the Lineweaver–Burke plot (Wright and Hobbie, 1966).

To determine T_t, acetate and formate uptake were each measured once in separate samples of winter precipitation. Five concentrations of ^{14}C-formate (final concentrations 4.7, 8.9, 13.0, 17.7 and 19.2 nM) and four concentrations of ^{14}C-acetate (final concentrations 14.0, 33.4, 73.2 and 141 nM) were added to 10 ml of sample (in triplicate) and allowed to incubate for 24 h at room temperature. After these incubations were stopped, the fractions (labeled cells and ^{14}C-CO_2) were prepared for liquid scintillation counting.

Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) (Nie *et al.*, 1975). The *t*-test was used to test for differences between sample mean turnover rate constants. All differences were considered significant at $P = 0.05$.

RESULTS AND DISCUSSION

Chemical analyses

The concentrations of formic and acetic acids ranged from 3 to 36 μM and 2 to 17 μM, respectively, over the study period (Table 1). Monthly average concentrations were weighted according to the volume of precipitation for each precipitation event. The lowest monthly volume-weighted average concentrations for both organic acids occurred in the winter, while the highest values occurred in the summer. The concentration of formic acid was 3.5 times that of acetic acid, and the correlation between formic and acetic acid concentrations ($r = 0.760$, $P < 0.001$) indicated a strong relationship.

Table 1. Volume-weighted average concentrations of chemical species (μM) and bacterial counts (cells ml^{-1}) as measured by Acridine Orange Direct Counts (AODC) in precipitation collected in Charlottesville, Virginia

Month	Rain (cm)	Formate (μM)	Acetate (μM)	Log AODC	pH
1983					
Nov	1.75	9.07	4.90	4.94	4.71
Dec	8.11	10.7	3.38	3.85	4.69
1984					
Jan	0.57	8.80	3.85	4.72	4.17
Feb	14.58	9.07	5.53	4.12	4.73
Mar	13.22	6.23	3.50	4.25	4.64
Apr	16.61	10.5	6.40	5.74	4.22
May	11.28	8.65	5.38	5.60	4.28
June	1.80	19.1	8.18	5.30	4.94
July	4.92	12.7	6.23	5.80	4.44
Aug	9.56	18.0	8.65	5.51	4.34
Sept	5.69	21.8	8.25	5.75	4.12
Oct	3.68	16.7	5.83	4.23	4.54
Nov	1.49	12.5	5.83	4.48	4.37
Dec	2.03	13.9	8.07	3.92	4.45

The formate and acetate concentrations in precipitation collected during this study are within the range of previously reported values (Galloway *et al.*, 1982; Likens *et al.*, 1983; Keene and Galloway, 1984a,b). Seasonally, the higher concentrations from April to September, observed in this study, fit the seasonal trend observed by Likens *et al.* (1983) and Keene and Galloway (1986). The observed peaks correspond to the growing season when more formate and acetate would be available to the atmosphere from plant sources (Babich and Stotzky, 1978; Hoffman *et al.*, 1980a, b; Graedel and Weschler, 1981; Keene and Galloway, 1986).

Microbiological analyses

Total number of bacterial cells. The monthly volume-weighted averages of direct cell counts (Table 1) agree with reported values in the literature and were an order of magnitude higher from April to September (2–6 $\times 10^5$ cells ml^{-1}) than during the rest of the year (0.8–5 $\times 10^4$ cells ml^{-1}). The cell counts reported for each event are the average of two subsamples, and the coefficient of variation associated with these averages ranged from 30 to 70%.

The literature on the seasonal distribution of airborne bacteria appears contradictory. In some studies, seasonal differences in concentration of bacteria were not observed (Fulton and Mitchell, 1966b; Wright *et al.*, 1969), whereas others report higher concentrations of airborne bacteria in either summer and spring, or summer and fall than other seasons (Lindeman *et al.*, 1982; Jones and Cookson, 1983). If the sources of bacteria to the atmosphere are considered, however, the data may be in more agreement. Bacteria may be released from living plant surfaces, plant litter or soils, all of which could have seasonal components (Lindeman *et al.*, 1982; Sands *et al.*, 1982). Bacteria-laden dust particles that become suspended due to human activity (i.e. traffic) would not necessarily have a seasonal component (Wright *et al.*, 1969; Bovallius *et al.*, 1978; Jones and Cookson, 1983). The studies that reported no seasonal effect were conducted in cities (Houston and Minneapolis-St. Paul), where airborne bacteria were assumed to come from human activity (Fulton and Mitchell, 1966a; Wright *et al.*, 1969). Seasonal effects were observed over agricultural fields in Wisconsin (Lindeman *et al.*, 1982) and a suburban area of Washington D.C. (Jones and Cookson, 1983), where vegetative sources are more abundant. The observed seasonal trend in the present study may have been caused by locally abundant vegetative sources of bacteria. The seasonal variation of bacteria also corresponded to the seasonal trend in formic and acetic

acid concentrations, which suggests that vegetation may be a common source for both microorganisms and these organics.

Biological uptake. The *in situ* activity of freshly collected microorganisms on formate and acetate was very low and was not significantly different from the controls (autoclaved precipitation samples). However, significant activity could be measured after the samples were incubated at room temperature for at least two days (Figs 1–3). Sterile controls (autoclaved rainwater) that were incubated under the same conditions as the samples did not have increased activity . The increase in activity in the samples coincided with an increase of one to two orders of magnitude in the number of bacteria (Fig. 4). Since sterile DIW that was poured through the funnels and incubated in the same manner did not show increases in either number of bacteria or heterotrophic uptake during the same time interval, the increase in total number of cells could only have been due to microorganisms that were present in the precipitation. The observed increase in cell number and activity was replicated in many precipitation events, with the large increase in uptake occurring between Days 1 and 4 (Figs 1–4). It is clear therefore, that precipitation contains microorganisms that are capable of utilizing formic and acetic acids. This observation alone is adequate to support the specu-

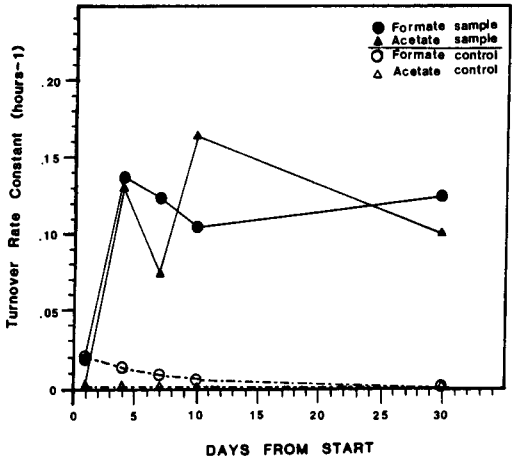

Fig. 1. Acetate and formate turnover rate constants (h^{-1}) at varying times after collection using rain collected on 28 April 1984. After collection, half of the sample was autoclaved and incubated at room temperature alongside the untreated sample to serve as a control. Cell numbers (cells ml^{-1}) in the rain samples were Day 1: 3.0×10^4; Day 4: 3.3×10^5; Day 10: 1.7×10^6. Cell numbers in the autoclaved control were Day 1: 4.5×10^3; Day 4: 1.7×10^4; Day 7: 1.0×10^4; Day 10: 1.3×10^4.

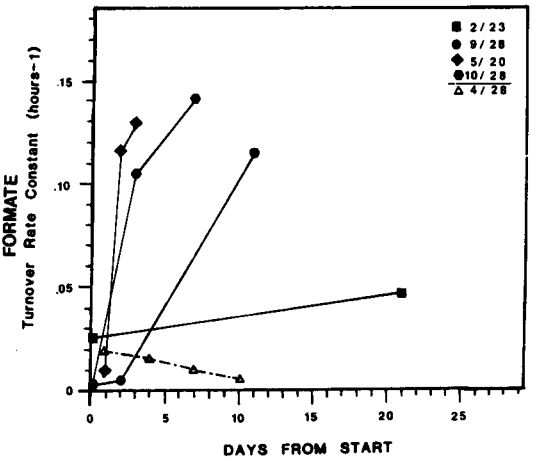

Fig. 3. Formate turnover rate constants (h^{-1}) measured at several time intervals after collection for four rain events in 1984. Samples were incubated at room temperature. Dotted lines represent autoclaved rainwater controls.

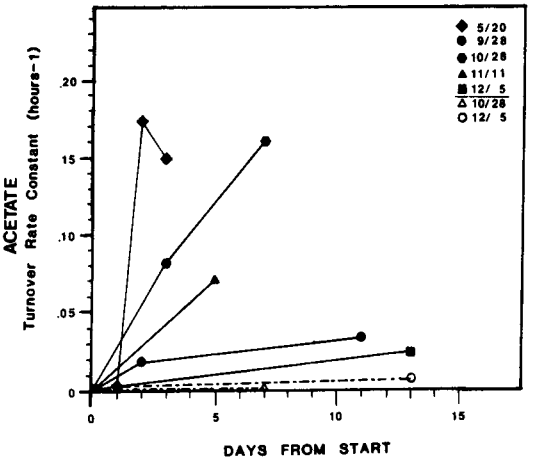

Fig. 2. Acetate turnover rate constants (h^{-1}) measured at several time intervals after collection for five rain events in 1984. Samples were incubated at room temperature. Dotted lines represent autoclaved rainwater controls.

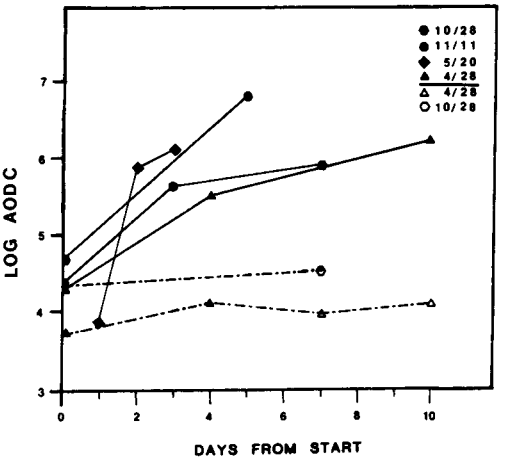

Fig. 4. Increase in cell numbers (cells ml^{-1}) with time in 1984 rain samples incubated at room temperature. Dotted lines are control rain samples that were killed by autoclaving. Note that the *y*-axis is a logarithmic scale.

lation of Keene and Galloway (1983, 1984a) that microbial mineralization and/or transformation is responsible for the observed loss of the acids in unpreserved samples.

Rates of uptake. Since the turnover rate constants for freshly collected precipitation samples were not different from the controls, the turnover rate constants that were measured after several days of incubation at room temperature (Figs 2, 3) (after the increase in cell numbers had occurred) were used. Maximum measured turnover rate constants of 0.17 and 0.14 h^{-1} for acetate and formate, respectively, multiplied by average substrate concentrations (6 μM acetate and 13 μM formate), show that losses of 24 and 44 μmol ℓ^{-1} of acetate and formate respectively could occur in one day. The actual amount of heterotrophic uptake of formate and acetate, in a freshly collected precipitation sample, is lower than these estimates, since cell populations were much lower than 10^6 cells ml^{-1} at the beginning of incubation.

Possible explanations of the low *in situ* activity on the day of collection are that the microbes were in resting phase while in the atmosphere or that other forms of dissolved organic carbon (DOC) are preferred and are used before formate and acetate. Other organic acids that have been identified in precipitation include butyric, succinic, lactic, propionic, valeric, glycolic and citric acids (Galloway *et al.*, 1976; Likens *et al.*, 1983; Keene *et al.*, 1983). Carboxylic acids, along with aldehydes, carbohydrates and tannin/lignin account for 31–41% of the total organic carbon and 83% of the low mol. wt DOC in precipitation at Hubbard-Brook and Ithaca (Likens *et al.*, 1983). Thus, many other sources of DOC are available to microorganisms in precipitation.

A second way of examining utilization rates was by using Michaelis–Menten uptake kinetics to calculate the turnover times. The calculated turnover times for acetate and formate show that the microorganisms were capable of 'removing' the natural pool of acetate in 34 ($\pm$ 18) days and formate in 1.5 ($\pm$ 2.3) days. Losses reported by Keene and Galloway (1983) can be explained by microbial activity. In this study however, acetate and formate were still present, even after 42 days of incubation. The fact that acetate and formate did not disappear from the samples may have been due to formate and acetate formation by other microbes in the sample, although this possibility was not examined. Both formate and acetate are known to be intermediate metabolic products and to accumulate in many bacterial suspensions (Quayle, 1972).

Of the total amount of ^{14}C-labeled formate that was utilized in all of the uptake studies, 95% was respired and 5% was assimilated, while 60% of the ^{14}C-acetate was taken up by the cell and 40% was respired. This wide difference reflects differences in efficiency of the organisms in deriving energy from the compounds, but it has implications for atmospheric chemistry in that respiration yields CO_2, whereas assimilation yields organic material (cells plus waste) that may conserve potential acidity in the atmosphere as the organics are further transformed.

In summary, the difference between the amounts of heterotrophic activity between the day of collection and several days of incubation is large. While the activity cannot be distinguished from controls on the day of collection, using the tracer method, activity after incubation can account for substantial losses of both formate and acetate.

CONCLUSIONS

This study has provided information about the relationship between microorganisms and formate and acetate concentrations in precipitation. Formate, acetate and bacterial concentrations varied seasonally, and were highest in the summer, corresponding to the time when the greatest amount of plant material is available to release bacteria to the atmosphere. The results of this study lead to the conclusion that precipitation contains microbes capable of mineralizing both acetate and formate. While it is clear that loss of these acids in unpreserved precipitation samples is a microbially mediated process, the data also suggest that low levels of activity may occur in the atmosphere, presumably in droplets. Seasonal variation in microbial abundance as reported here, coupled with seasonal variation in organic acid content as reported elsewhere, implicates vegetative sources for both the microbes and their substrates. Future work should address the question of microbial activity in the atmosphere in terms of both organic acid production and consumption.

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