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Effects of Atmospheric Humidity and Temperature on the Survival of Airborne *Flavobacterium*

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The survival of airborne *Flavobacterium* sp. in particle sizes ranging from 1 to 5 μm was significantly influenced by atmospheric temperature. A progressive increase in temperature from −18 to 49 C resulted in increases in death rates of the airborne organism. The lowest death rates were observed in the temperature range of −40 to −18 C, and the highest death rates were observed in the 29 to 49 C range. At 24 C, the survival of airborne *Flavobacterium* did not appear to be significantly affected by relative humidity ranging from 25 to 99%.

A *Flavobacterium* sp. isolated from a water supply source was reported by Won and Ross (8) to show aerosol survival characteristics (at −30 C) similar to those of *Bacillus subtilis* var. *niger* spores. The stability of airborne *B. subtilis* spores has made their inclusion in slurries of vegetative microorganisms a convenient and sensitive method for separating physical from biological phenomena, during studies of the effects of environmental stresses on airborne microorganisms. In our experience, a variety of vegetative microorganisms have not exhibited the high resistance to environmental stresses of temperature and humidity associated with spores of *B. subtilis* (4). Studies were conducted in our laboratories to examine the performance of airborne *Flavobacterium* sp. in a static aerosol chamber at various conditions of relative humidity (RH) and temperature. If the stable nature of the microorganism reported by Won and Ross could be confirmed, the organism would merit additional study to examine the biophysical and biochemical mechanisms responsible for its aerobiological stability and to determine its potential use as a physical tracer morphologically similar to less stable vegetative microorganisms.

MATERIALS AND METHODS

Frozen, concentrated stock cultures of *Flavobacterium* sp. and spores of *B. subtilis* (batch no. 91) were stored at dry ice temperature until used. The *Flavobacterium* was grown as described by Won and Ross (8). The microorganisms were thawed, and individual suspensions, consisting of 7.0 g of *Flavobacterium* and 11.5 g of *B. subtilis* each, were prepared in 100 ml of gelatin phosphate diluent. The suspensions were blended in a Waring Blendor and filtered through an 80-mesh stainless-steel screen. For dissemination, fresh mixtures of the two agents were prepared daily. The intimate mixtures consisted of 9.0 and 1.0 ml of the *Flavobacterium* and *B. subtilis* suspensions, respectively, resulting in a 10 to 1 ratio of the vegetative microorganism to the spore tracer. Based on previous experience, this ratio provided a sufficiently high concentration of *B. subtilis* spores in the aerosol to permit a meaningful quantitative assay. At the same time, because of the intimate nature of the mixture, decrease in aerosol concentration of *B. subtilis* spores reflected the physical decay of both microorganisms.

For dissemination by means of a two-fluid atomizer (FK-8 aerosol gun), 1.0 ml of the mixture was used. The atomizer produced an aerosol having a majority of particles in the 1- to 5-μm size range. The aerosol was produced in a 2,500-liter Freon-tight stainless-steel aerosol chamber. To maintain a uniform distribution of the aerosol, a small fan was continuously operated in the chamber during the experimental trials (4).

The airborne bacteria were collected for 1 min at 4-, 16-, 32-, and 64-min cloud ages with two parallel all-glass impingers [AGI-30 (1)] operated at a sampling rate of 12.5 liters per min. A single-stage impactor (5), designed to remove particles larger than 5 μm, replaced the usual curved stem of the AGI-30 sampler. The impingers contained 20 ml of gelatin phosphate diluent with 0.15% Dow-Corning Antifoam B emulsion. To prevent freezing of the collecting fluid during experiments involving subfreezing temperatures, the AGI-30 samples were maintained in a water bath at approximately 20 C.

For quantitative assay, the contents of the duplicate AGI-30 samplers were pooled, and the viable bacteria were enumerated by standard bacteriological dilution and plating techniques. *Flavobacterium* was assayed on Casitone agar (Difco) containing, per milliliter, 0.03 μg of Brilliant Green and 0.1 mg of cycloheximide used as inhibitors of *B. subtilis* and fungal contaminants, respectively. *B. subtilis* was assayed on Tryptose Agar containing, per milliliter, 3.0 μg of potassium telurite and 0.1 mg of cycloheximide as inhibitors of the vegetative organisms and fungal contaminants, respectively. Results of exploratory studies conducted with the inhibitors indicated that they had no effect on the growth of the two microorganisms which were to be assayed. The plates were incubated at 37 ± 1 C for 48 hr, and the bacterial colonies were counted.

The desired humidity and temperature conditions in the aerosol chamber were established before dissemination of the bacteria and were maintained and monitored throughout each aerosol trial (4). The humidities investigated during the studies were 25, 45, 65, 85, and 99 ± 5% RH at 24 ± 2 C. The temperatures studied were −40, −18, −2, 24, 29, 38, and 49 ± 2 C. At −2 C and above, the humidity in the chamber was maintained at 85 ± 5% RH, whereas at −18 and −40 C ambient, essentially saturated atmosphere was used.

Whenever applicable the mean estimates of the aerosol parameters were compared by the standard analysis of variance technique. The means were based on eight replicate aerosol trials conducted at each temperature or humidity condition. The significance of the differences observed among the means was determined at the 5% probability level.

RESULTS AND DISCUSSION

To define the effects of RH and temperature on airborne *Flavobacterium* (particles 1 to 5 μm in size), three parameters were used. First, the biological death rate, expressed in per cent per minute (%/min), represented the difference between the rate of loss of viable vegetative cells from the aerosol (total decay rate) and the rate of loss due to physical decay as indicated by the *B. subtilis* spores. This value defined the death rate of airborne *Flavobacterium* under a given set of experimental conditions during the 64-min aerosol age. Second, the recovery of viable cells from the aerosol, expressed as percentage of the total quantity of *Flavobacterium* disseminated, provided information on the recovery of the organisms at 4, 16, 32, and 64 min after the dissemination. Third, the per cent survival, based on the ratio of *Flavobacterium* cells to *B. subtilis* spores initially present in the disseminated mixture (representing 100% recovery) and those present in the aerosol samples, defined the viability ratio. Previous studies (4) indicated that no significant losses in viability of *B. subtilis* spores were observed within the temperature and humidity conditions used in present experiments. Thus, this analysis provided survival data for *Flavobacterium*, which was not affected by variables such as efficiency of dissemination and sampling and settling of the aerosol particles.

Relative humidity. Table 1 summarizes the death rate and recovery of viable *Flavobacterium* cells from aerosols at various humidity conditions at 24 C. Statistical analysis of the data indicated that the only significant differences among the estimates of death rate occurred at 85% RH (1.27 ± 0.21%/min) and 99% RH (2.61 ± 1.42%/min). However, in the analysis of variance the value of F was 2.94, being on the borderline of significance.

The recovery of viable *Flavobacterium* cells from aerosol at 24 C was not significantly influenced by humidity ranging from 25 to 99% RH. Whereas the aerosol recovery tended to decrease with a progressive increase in RH, a parallel change was seen in the recovery of *B. subtilis* spores used as the physical decay tracer. This would suggest some effect of RH on the physical, rather than the biological, characteristics of the aerosol. The decreased recovery of organisms from the aerosol at the extremely high RH could be due, in part, to the equilibration of airborne particles to a mass median diameter larger than 5 μm.

In general, airborne *Flavobacterium* appeared to be less sensitive to RH than other vegetative microorganisms. Bacteria such as *Escherichia coli* and *Serratia marcescens* have been reported

TABLE 1. *Recovery and death rate of airborne Flavobacterium at various relative humidities at 24 C*

RH[a] (%)	Cloud age				Death rate (%/min)
	4 min	16 min	32 min	64 min	
25	28.1[b]	16.8	9.4	3.9	1.72
45	30.5	20.1	11.4	5.2	1.73
65	24.1	13.2	7.3	3.5	1.46
85	25.8	15.7	9.2	4.9	1.27
99	23.8	10.4	4.1	1.6	2.61

[a] RH, relative humidity.

[b] Per cent viable recovery at cloud age.

TABLE 2. *Death rate of airborne Flavobacterium at various temperatures*

Temp (C)	RH[a] (%)	Death rate (%/min)
−40	Ambient[b]	0.01
−18	Ambient[b]	−0.10
−2	85	1.52
24	85	1.72
29	85	3.04
38	85	3.70
49	85	4.69

[a] RH, relative humidity.

[b] Saturated atmosphere.

to show critical RH levels at which significantly lower cell survival in aerosols was observed (2, 3, 6, 7). Such a relationship between RH and survival was not observed for the airborne *Flavobacterium*. However, at all RH levels, *Flavobacterium* showed a significantly lower survival in aerosols than spores of *B. subtilis* disseminated as an intimate mixture.

Temperature. Table 2 shows the mean death rates of *Flavobacterium* aerosolized at various temperatures. The death rate of this microorganism appeared to increase upon progressive increase in temperature from −18 to 49 C, and the data suggested three levels of response to temperature. The first level was the temperature ranging from −40 to −18 C within which losses in viability were not apparent. The second level was the range between −2 and 24 C within which the mean death rate was 1.62 ± 0.72%/min. The third level included temperatures ranging from 29 to 49 C resulting in a mean death rate of 3.81 ± 1.20%/min. The differences between the death rates observed at the three levels were significant.

The relationships between the death rate of *Flavobacterium* and the temperature is further illustrated in Fig. 1. The survival of this organism in the aerosol was calculated on the basis of the ratios of *Flavobacterium* to *B. subtilis* spores in the disseminating suspension (representing 100% survival) and those in the aerosol samples collected at various cloud ages.

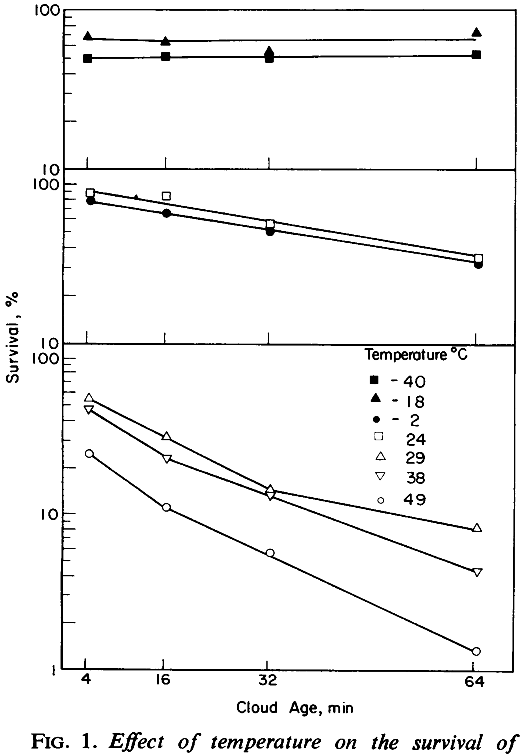

FIG. 1. *Effect of temperature on the survival of airborne Flavobacterium sp.*

From the data, it can be concluded that the survival of airborne *Flavobacterium* was significantly influenced by the atmospheric temperature. A progressive increase in temperature from −18 to 49 C resulted in a reduced survival of this microorganism in aerosol. The increased death rates and reduced survival of airborne *Flavobacterium* at 49 C was not surprising. This temperature is near the critical point of thermal inactivation of bacteria and one at which some alterations in the proteinaceous structure and enzymatic activity can be expected. The reduced survival of *Flavobacterium* at 29 as compared to 24 C, also observed in *S. marcescens* and *E. coli* (4), again emphasizes the necessity of close temperature control in studies of airborne microorganisms.

Results of our studies are in agreement with those reported by Won and Ross (8) in that, at −40 C, the *Flavobacterium* manifests survival in aerosols approximately equal to that of *B. subtilis* spores. However, this does not appear to be true of the other higher temperatures studied. For example, at 24 C the death rate of *Flavobacterium* was almost identical to that of *S. marcescens* and *E. coli*, but there was no detectable biological decay of *B. subtilis* spores. At 49 C the death rate of *Flavobacterium* was approximately one-third that of the other two vegetative microorganisms. However, at this temperature the death rate of *Flavobacterium* was 10-fold that of *B. subtilis* spores [4.7%/min versus 0.49%/min (4)]. Thus, the results of our experiments indicate that airborne *Flavobacterium* sp. does not have the characteristics required of a physical tracer for aerosol studies.

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