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Effect of Diluent and Relative Humidity on Apparent Viability of Airborne *Pasteurella pestis*

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ABSTRACT

WON, WILLIAM D. (University of Caifornia, Berkeley), AND HAROLD ROSS. Effect of diluent and relative humidity on apparent viability of airborne *Pasteurella pestis*. Appl. Microbiol. **14**:742–745. 1966.—Airborne *Pasteurella pestis* (A-1122) at low humidities [20 to 50% relative humidity (RH)] exhibited exponential decay when either 1% peptone or Heart Infusion Broth (HIB) was used as the diluent in the viable assay system. At higher RH values (65 and 87%), however, the 1% peptone diluent adversely affected the viability assay. In contrast, HIB as diluent was remarkably effective in demonstrating a higher number of viable cells in aerosols held at high RH values. Similarly, with HIB as diluent, aerosols were shown to contain viable cells during 90 min of observation; with 1% peptone, viability was not detectable after 20 min in the airborne state.

Relative humidity (RH) has striking biological effects on microorganisms. Dunklin and Puck (3) and Webb (5) reported that the death rate for aerosolized *Serratia marcescens, Escherichia coli, Staphylococcus albus, Streptococcus hemolyticus, Bacillus subtilis* and type 1 pneumococci was greatest at the intermediate RH zone. On the other hand, increased death rate due to exposure to high RH environments has also been observed for airborne *Salmonella pullorum* and smears of *Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, and Salmonella derby* (1, 4). This report describes results of experiments with *Pasteurella pestis* (A-1122), showing that diluents used in viable-cell assay of aerosol samples have an effect on the apparent viability of organisms aerosolized and maintained at various RH values.

MATERIALS AND METHODS

P. pestis cultures. The avirulent strain A-1122 was grown as second-passage culture for 24 hr at 37 C in 200 ml of Heart Infusion Broth (HIB) contained in a 500-ml Erlenmeyer flask on a gyrotory shaker at 200 oscillations per min. Viability in these cultures, as determined by 10-fold serial dilution in 1.0% peptone broth, consistently assayed at approximately 2×10^9 organisms per milliliter.

Stirred settling chamber. Description and procedure for the operation of the apparatus have been given (2). In lieu of the original cubical chamber, however, the unit used here was a cylinder measuring approximately 1.0 meter in diameter and 1.5 meters in height, with a contained volume of 1,200 liters. It was equipped with glove and viewing ports but did not have a temperature-regulatory mechanism. A motor-driven fan, mounted on the bottom, provided stirred settling conditions within the tank.

For creating and maintaining an atmosphere of a particular RH, water-saturated air was mixed with dry air and introduced into the chamber. The RH was checked by means of a self-circulating Weksler-type hygrometer within the unit.

Bacterial aerosols. Cultures were centrifuged at 3,020 × *g* at 4 C for 10 min, after which period the cells were resuspended in 50 ml of the supernatant fluid. Such suspensions generally contained between 10^{10} and 2.0×10^{10} cells per milliliter. The suspension was held at 4 C until use. For each experiment, 25 ml of the concentrated suspension was used in a Wells-type atomizer operated with dry air at 20 psi for 10 min, giving a total fluid output of 2.3 ml.

Aerosol sampling. At set intervals, samples were collected in glass impinger samplers, AGI-30 (7), containing 20 ml of HIB supplemented with 0.1% Dow Corning antifoam B. Sampling time was 1 min at a flow rate of 12.5 liters per min. The number of viable cells was determined by preparing 10-fold serial dilutions in 1% peptone (or other media; see Results) and plating 0.1 ml of the appropriate dilutions on Difco Blood Agar Base medium. In some instances, where low numbers of viable cells were expected, 0.25- or 0.5-ml quantities were used for plating. Inoculated plates were incubated for 48 hr at 37 C.

RESULTS

Data shown in Table 1 are typical of the number of viable cells obtained with *P. pestis* a-1122 aerosol samples after exposure to 87% RH at ambient temperature (26 C), when the conventional

TABLE 1. *Influence of diluent on viability pattern of Pasteurella pestis A-1122 aerosols exposed to 87% relative humidity (26 C)*

Age of aerosol	Colony-forming units per plate[a]			
	Undiluted HIB collecting fluid	Dilution with 1% peptone		
		10^{-1}	10^{-2}	10^{-3}
min				
0[b]	TNTC[c]	TNTC	TNTC (>1,000 colonies/plate)	0, 17, 4[d]
5	TNTC	TNTC (>1,000 colonies/plate)	46, 77, 59	1, 0, 2[d]
10	TNTC	365, 378, 390	0, 1, 1[d]	
20	TNTC (>1,000 colonies/plate)	12, 1, 0	0, 1, 0[d]	
30	TNTC (>1,000 colonies/plate)	0, 0, 7	0, 0, 0	
50	TNTC (>1,000 colonies/plate)	4, 0, 1	0, 0, 0	

[a] Inocula of 0.25 ml of each dilution were used with aerosols of age 0, 5, and 10 min, and 0.5 ml was used with aerosols of age 20, 30, and 50 min.

[b] Zero-time commenced immediately after termination of cloud dispersal.

[c] Too numerous to count.

[d] Continued incubation for an additional 24 hr did not show an increased number of colonies.

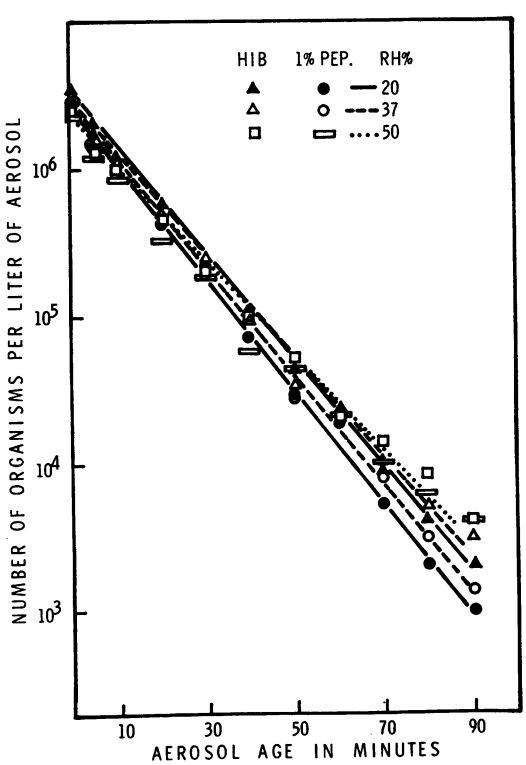

FIG. 1. *Effect of diluent and relative humidity on viability of aerosolized Pasteurella pestis A-1122.*

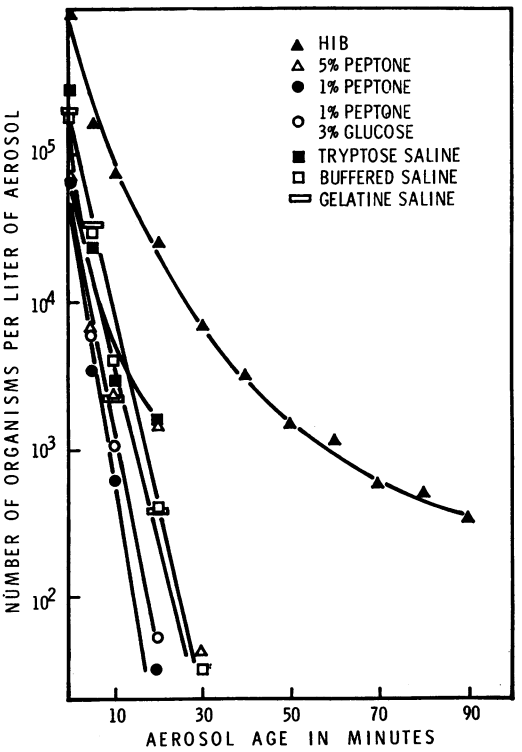

FIG. 2. *Effect of diluent on viability of aerosolized Pasteurella pestis A-1122 maintained at 87% RH (26 C).*

1% peptone was used for preparing the serial dilutions. These results indicated an accelerated death rate, and at the same time showed disproportionate counts among the triplicate plates and between the stepwise dilutions. Similar results were observed with aerosols exposed to 65% RH. Behavior of airborne cells held at RH values below 50%, subsequently collected and diluted in 1% peptone or HIB, is seen in Fig. 1. At these low RH values, decay rates were essentially identical and were independent of the diluent used. Similarly, the pattern of disproportionate yields of viable cells and accelerated death rates, typical with 65 and 87% RH aerosols diluted in peptone, was consistently absent under low RH conditions.

The relationship between diluents containing increased concentrations of peptone and those prepared with materials other than peptone, when used for the dilution of aerosol samples previously exposed to 87% RH, is shown in Fig. 2. Even a simple inorganic diluent (buffered saline) was shown to be superior to the 1% peptone solution. However, some improvement of the conventional 1% peptone diluent resulted when it was supplemented with 3% glucose, or when the peptone concentration was increased to 5%. Apparently, an increased concentration of peptone was more effective than supplementation with glucose. The highest number of viable cells in the sampler fluids was obtained when HIB was used as the diluent. Unlike other samples, those diluted in HIB yielded the expected proportionality of counts as a function of dilutions. Furthermore, the use of HIB as the diluent permitted detection of viability in aged aerosols at high RH. At an aerosol age of 90 min, sampler fluid diluted with HIB indicated an aerosol concentration of about 3×10^2 viable organisms per liter, whereas the same fluid diluted with 1% peptone and other diluents gave no indication of viable cells. These results were obtained in five experiments. Similar results were found in parallel experiments conducted with a second avirulent strain of *P. pestis* designated as EV-76.

DISCUSSION

Our studies demonstrated that the diluent and high RH influence the apparent survival of aerosolized *P. pestis* A-1122. The RH examined in this study included 20, 37, 50, 65, and 87% at ambient temperatures (26 C). At low humidities (20 to 50%), the bacterial cloud evinced a continuous biological decay at a constant rate, independent of the type of diluent used. In contrast, aerosols exposed to atmospheres of high RH (65 and 85%) exhibited an abrupt loss of viable organisms when sampler fluids were serially diluted with 1% peptone. Furthermore, viability estimates became erratic and lacked proportionality between dilutions.

In aerosol formation, airborne cells are coated with materials contained in the suspending menstruum (6). At high RH, it is conceivable that sufficient water is present in this microenvironment to permit metabolic functions which lead to alterations in permeability of the cell membrane. On transfer to a new environment, the cells may be unable to control the flux of material transfer across the membrane. If the new environment contains materials which qualitatively and quantitatively are lethal, the cell is "dead." It would seem that peptone contains both lethal and protective materials, and it is the absolute amount of each (see results for 1% and 5% peptone) which determines the outcome.

The other diluents used (except for HIB) apparently lacked the lethal elements present in peptone; HIB perhaps contains, in balance, more of that which protects.

Whatever the mechanism involved, plating of the organisms in the undiluted collecting fluid, HIB, consistently produced plates with too many colonies to be counted. If the undiluted collecting fluid had not been plated, the inhibitory effect associated with the 1% peptone diluent would easily be overlooked, and the rapid decay rate would have been erroneously considered to be a direct function of high RH. Previously, it was not suspected that the conventional 1% peptone could exert such a significant detrimental influence. In view of greater recovery of airborne organisms exposed to high RH with modified peptone and HIB diluents, one wonders if there is not an even more efficient growth substrate and sample diluent for supporting growth of greater numbers of potentially viable cells. Similarly, some thought should be given to the validity of respiratory LD_{50} values for these airborne organisms previously determined with the use of 1% peptone-water as the assay diluent.

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