RELATIVE HUMIDITY AND THE KILLING OF BACTERIA

I. OBSERVATIONS ON Escherichia coli AND Micrococcus lysodeikticus

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Some observations on the ability of washed Escherichia coli and Micrococcus lysodeikticus to form colonies when plated after having been freeze-dried and exposed to water vapor in vacuo will be presented in this paper. Serratia marcescens treated in this manner is rapidly killed within a rather narrowly limited range of relative humidities around 90 per cent (Monk and McCaffrey, 1957). It was of interest to determine whether other organisms would behave similarly.

MATERIALS AND METHODS

E. coli. Resting cell suspensions of E. coli ATCC strain 4157 were made by harvesting the growth from Roux bottles on nutrient agar incubated at 37 C for 18 to 24 hr. After rinsing the cells from the surface with distilled water and centrifuging, the stock suspension was obtained by resuspending the sediment in enough distilled water to give about 10^11 viable cells per ml.

The suspensions were freeze-dried in 0.5-ml portions for 1 hr in the glass inverted U manifold described by Monk and McCaffrey (1957) and equilibrated in vacuo at relative humidities of 0, 11, 75, or 93 per cent. At intervals from 3/2 to 5 hr tubes were detached from the apparatus and the contents diluted with 0.5 ml of water and plated after serial dilution. In each experiment at least one tube was reserved for dry weight determination.

M. lysodeikticus. The stock suspensions were prepared as described for E. coli and the same procedures of freeze-drying and hydration were used.

RESULTS

E. coli. In the results shown in figure 1, exposures to 0 and 93 per cent relative humidity were made simultaneously on cell samples from a single stock suspension. Presence of water

Figure 1. Death of freeze-dried washed Escherichia coli stored in vacuo under different conditions. Abscissa, time of storage in hours. Ordinate, logarithm of fraction of initial viable count. Open circles: 0 per cent relative humidity; heavy circles: 93 per cent relative humidity. All data obtained simultaneously with a single cell preparation divided among the specimen tubes of the Monk-McCaffrey (1947) manifold.

Figure 2. Death of freeze-dried washed Escherichia coli stored in vacuo under different conditions. Abscissa, time of storage in hours. Ordinate, logarithm of fraction of initial viable count. The different sets of data are identified by numbers giving relative humidity and temperature, in centigrade, respectively. Lines are drawn by inspection. Data were obtained at different times with different weekly stock cell suspensions.

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vapor at relative humidity of 93 per cent clearly results in more rapid destruction of bacteria than in its absence. Both curves are approximately exponential over a 3-hr exposure period.

Additional data are reported in figure 2; these represent separate, rather than simultaneous, exposures to different conditions. Again a clear distinction exists between the initial rates of destruction at 0 and 93 per cent relative humidity at room temperature; after 3 hr the 93 per cent rate tends to level off; the points at 24 hr show, however, that killing continues, although at a reduced rate. Limited data obtained at 11 and 75 per cent relative humidity values are omitted from figure 2; neither set of data is distinguishable with certainty from that at 0 per cent relative humidity. Data obtained at 37 C with 75 and 93 per cent relative humidity are included; the initial death rates are clearly greater than those at 25 C, but the curves are not exponential, and for exposure times of 1 to 3 hr no temperature effect is discernable. Broth causes a very large acceleration in rate of killing at 93 per cent relative humidity and room temperature.

*M. lysodeikticus*. Freeze-dried and hydrated, washed *M. lysodeikticus* proved to be very stable. At "zero" relative humidity no loss of viability could be detected over 4½ hr. The data at 11 per cent relative humidity (table 1) also show very little loss in viability in 24 hr. At 93 per cent relative humidity (table 1) the same is true for 4 hr, whereas in 24 hr, a more definite loss occurred with an over-all first order constant not greater than 0.1 hr⁻¹.

**Discussion**

The effects of water vapor in vacuo on washed *S. marcescens* and *E. coli* are qualitatively similar within the limits of the present comparison: moisture is toxic to both organisms, and a relative humidity of 93 per cent produces in each case more rapid killing than relative humidity values of 75, 11, or 0 per cent. *E. coli* is, however, the less sensitive of the two organisms at 93 per cent relative humidity. *M. lysodeikticus* on the other hand is very stable; at 93 per cent relative humidity for instance, the relative viable count after 24 hr is 400,000 times greater than in the case of *E. coli*. These results are in line with the widespread impression (Fry, 1954) that some of the cocci are much less sensitive to drying than many gram-negative organisms.

Only very limited comparisons of these results with aerosol stability data are possible. The recent extensive work of Hemmes (1959) on aerosols produced from cell suspensions in presence of 1 per cent peptone, shows, at all relative humidity values, the greater stability of streptococci and of *Staphylococcus aureus* in comparison with *S. marcescens* and *E. coli*, in agreement with the contrast of *M. lysodeikticus* to *E. coli* and *S. marcescens* shown in our results. According to his results both *S. marcescens* and *E. coli*, however, become increasingly sensitive as the humidity is decreased, with a rather steep increase of rate of killing around 60 per cent relative humidity and a very broad maximum at about 30 to 50 per cent. Obviously the effects of air and of extrinsic solutes complicate these results.

If selective humidity effects on cell viability are to be interpreted in terms of the interplay of water, solutes and cell structures, as suggested elsewhere (Bateman, 1959 unpublished data), the basis for the differences in stability between the cocci and the gram-negative organisms might very well lie in the relatively high intracellular solute concentration in the cocci. (Dr. W. J. Scott of the Commonwealth Scientific and Industrial Research Organization, New South Wales, expressed the opinion in a conversation in 1958 that the stability of the staphylococci

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**TABLE 1**

<table>
<thead>
<tr>
<th>Viable Cells after Exposure of Hr:</th>
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<tr>
<td>Relative humidity = 11%, water* 12%</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>3.1</td>
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<tr>
<td>6.5</td>
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* Water content of cells, obtained by measurement of weight loss on drying.
was directly due to their amino acid pool.) These solutes consisting largely of amino acids with functional groups common to those of the cell macromolecules, may well be supposed to protect sensitive sites of the macromolecules from the effects of other toxic solutes, by competitive interaction.

SUMMARY

Water vapor is lethal to freeze-dried washed Escherichia coli held in a vacuum; the rate of killing at 93 per cent relative humidity is considerably greater than at lower humidities. This property E. coli possesses in common with Serratia marcescens, although to a smaller degree, whereas Micrococcus lysodeikticus under the same experimental conditions is many times more stable and was not shown to be selectively killed at 93 per cent relative humidity.

REFERENCES

