The Survival of *Escherichia coli* in Nitrogen Atmospheres under Changing Conditions of Relative Humidity

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SUMMARY

The consequence of relative humidity (RH) changes before collection of bacterial aerosols are described; it is shown how *Escherichia coli* (B, commune), but not *E. coli* Jepp, gave good survival over the range 100–20 % RH provided that the following conditions applied: (1) a pure nitrogen atmosphere; (ii) a protective agent in the spray fluid; (iii) sucrose in the collecting fluid; (iv) an RH change to 100 % or to a nominal 30 % (depending upon the experimental conditions) before collection of the aerosol. Differences in survival of *E. coli* commune sprayed from suspension in solutions of raffinose into an atmosphere of nitrogen which were previously attributed to the effect of raffinose concentration in the spray fluid were found to be caused by the use of variant organisms.

INTRODUCTION

Previous papers (Cox, 1966; Cox & Baldwin, 1966) showed that the survival of *Escherichia coli* as an aerosol was greatly enhanced at low relative humidity (RH) when nitrogen replaced air. In the high RH range, regions of marked aerosol instability occurred for the bacteria sprayed from suspension in distilled water, while the addition of a protective agent altered both the size and the RH value of the minima in plots of survival versus RH. The possibility was considered that a shift in RH from high (unstable) to low (stable) values before collection might indicate whether the death of *E. coli* occurred in the aerosol at high humidity or as a result of the collection technique. The present paper gives the results obtained and shows the extent to which decrease of survival values may be attributed to the method of sampling. In previous work (Cox, 1966) the effect of the protective agent appeared markedly to depend on its concentration for *E. coli* commune, whereas for *E. coli* (B, Jepp) the effect was much less marked. The possibility that the apparent effect of concentration of protective agent might have been associated with a change of stock culture was also examined, even though different stock cultures gave suspensions having very similar survival characteristics under the conditions of test when sprayed from distilled water.

METHODS

The techniques used were as previously reported (Cox, 1966), with the addition that chambers of 2 l. capacity as in Fig. 1 were used to bring about the changes in the RH shifts. Partial filling of the chambers and wetting of the muslins, with

distilled water or with saturated lithium chloride (LiCl_2) solution, enabled RH values of approximately 100 and 30 %, respectively, to be achieved. The value of 30 % RH is in doubt since this was the value achieved when 12 l. wet nitrogen/min. flowed through the chamber. In equilibrium conditions an RH value of 12 % should be achieved; since the aerosol cloud remained static in the chambers for 30 sec. before collection the true value of RH will have been between these two extreme values. This RH range corresponded to a plateau region of good survival for *Escherichia coli* in nitrogen (Cox, 1966). The chambers were maintained under an atmosphere of nitrogen (> 99.9 %) at all times.

Fig. 1. Chamber of 2 l. capacity used for making relative humidity changes.

RESULTS

Figure 2 shows the survival of *Escherichia coli* commune 2 when sprayed into nitrogen from 0.13 M-raffinose in distilled water (added to the bacteria immediately before spraying), at an aerosol age of 25 min. for collection by raised impinger (May & Harper, 1957) into phosphate buffer and into M-sucrose + phosphate buffer. Similar to previous results (Cox, 1966) collection in phosphate buffer was marked by instability within the range 72–86 % RH, whereas collection in M-sucrose + phosphate buffer revealed only one clear minimum at RH 81–82 %. However, with this variant of *E. coli* commune the extent of the instability was less than that previously observed.

Figures 3 and 4 show the effect of changing the RH of the nitrogen atmosphere either to 100 % or to 30 % before collecting the aerosol. In general phosphate buffer and M-sucrose + phosphate buffer gave similar results outside the unstable zone of 72–86 % RH. In the unstable zone phosphate buffer remained inferior to M-sucrose + phosphate buffer when the shift was to 30 % RH, but the difference was smaller when the shift was to RH 100 %. Suspensions prepared from a third stock culture of *Escherichia coli* commune gave similar results under static and changing RH conditions.

Table 1 includes results for three strains of *Escherichia coli* when sprayed into nitrogen from distilled water and from 0.13 M-raffinose in distilled water (added to the bacteria immediately before spraying) at an aerosol age of 25 min. The test RH values were those corresponding to minima in the plots of survival versus RH, or were at 30 % RH where the stability was much greater. The data agree with that

previously published (Cox, 1966) and show that when sprayed from distilled water RH changes before collection did not markedly effect the survival of *E. coli* (B, Jepp) stored at RH 85 %. However, for *E. coli* commune 2 at RH 81 % a change to 100 %, but not to 30 %, produced a large increase in the survival in both collecting fluids. When the organisms were sprayed from raffinose the pattern of results was

Figs. 2-4. Survival of *Escherichia coli* commune 2 in nitrogen, sprayed from 0.13 M-raffinose at an aerosol age of 25 min. ●, Collection in phosphate buffer; ○, collection in M-sucrose + phosphate buffer. Fig. 2. At the storage relative humidity. Fig. 3. At the storage relative humidity followed by a change in relative humidity to 100 % before collection. Fig. 4. At the storage relative humidity followed by a change in relative humidity to 30 % before collection.

more complex. A change from RH 83 to 100 %, but not to 30 %, eliminated the difference between collection in the two fluids for *E. coli* B and gave 100 % survival. For *E. coli* Jepp neither a change to RH 100 % nor to 30 % gave enhanced survival for either collecting fluid. *E. coli* commune 2 gave 100 % survival when the RH changed to 100 %, but the change to 30 % resulted in survival being dependent upon collecting fluid.

For aerosols stored at a controlled RH value of 30 % no really marked effect of changes of RH occurred for *Escherichia coli* B and commune 2. However, for *E. coli* Jepp, as might be expected from its instability at high RH (Cox, 1966) a change to RH 100 % caused a rapid decrease of survival. The results for *E. coli* Jepp also showed the previously reported (Cox, 1966) poorer survival in M-sucrose + phosphate buffer than in phosphate buffer alone which occurred at low RH, but not at high RH; these results contrast with those obtained with *E. coli* B and commune at low RH.

Table 1. *The influence on stored aerosols of Escherichia coli in nitrogen at 26.5° of relative humidity changes before collection*

Strain of <i>E. coli</i>	Spray fluid	Percentage RH for storage	Percentage survival					
			At storage RH		At 100% RH		At 30 % RH	
			PB*	PBS*	PB	PBS	PB	PBS
B	D†	85	0.5	0	0	2	0	0
		30	80	77	65	35	85	61
Jepp	D	85	0	0.5	0.9	0.4	0	0.2
		30	50	7	9	0.9	42	3
commune 2	D	81	3.3	6	18	21	1	4
		30	84	82	83	70	82	90
B	R‡	83	39	102	100	100	38	100
		30	110	113	124	127	104	105
Jepp	R	82	2.5	15	1	2	5	15
		30	97	22	64	28	94	25
commune 2	R	82	77	73	99	100	66	108
		30	94	100	115	114	104	117

* PB = phosphate buffer collecting fluid; PBS = M-sucrose + phosphate buffer collecting fluid.

† D = distilled water. ‡ R = 0.13 M-raffinose in distilled water added immediately prior to spraying.

DISCUSSION

The difference in behaviour of *Escherichia coli* commune when sprayed from 0.13 and 0.3 M-raffinose, located outside the cell wall, was previously attributed (Cox, 1966) to the difference in raffinose concentration. From the results of the experiments given in the present paper it now seems to have been caused by the use of stock cultures which contained different variants of *E. coli* commune. These variants were not distinguishable under the conditions of test in aerosol behaviour when sprayed as a suspension in distilled water, but were distinguishable in the presence of raffinose. These findings are perhaps an indication of the subtlety of the action of raffinose as a protective agent. As far as can be judged, each stock culture gave rise to suspensions of bacteria showing consistent behaviour, and it has been possible to achieve similar results for suspensions arising from different stock cultures. The three stock cultures all gave suspensions of bacteria which, when sprayed from raffinose, showed the same trends, namely, good survival at high and low RH and poorer survival in the RH 72–86 % region, with minima at 74 and 78 % (and at 81 % RH for one culture, others not tested at this RH). Hence it was the degree of response rather than the response itself which was dependent upon the stock culture

used. This difference in stock culture so far has not been found for *E. coli* B and Jepp. Dr G. J. Harper (personal communication) has found that *E. coli* commune gives variants.

Previous work (Cox, 1966) showed that part of the survival decrease of *Escherichia coli* occurred during or following collection by raised impinger (May & Harper, 1957). Transfer from the unstable relative humidity zone to a stable zone before collection should indicate the proportions in which death occurred in the aerosol and in the impinger. The stable state most easily obtained for *E. coli* (B, Jepp, commune) was in the region of RH 30 %. A second region near RH 100 % was reasonably stable for bacteria protected with raffinose, but not when sprayed from distilled water alone. The results (Table 1) show that a shift to RH 100 % usually increased the survival of *E. coli* B, commune but not of *E. coli* Jepp. A shift to RH 30 % had no significant effect for *E. coli* B and Jepp with either spray fluid; however, for *E. coli* commune sprayed from raffinose, but not distilled water, the shift in RH was beneficial.

Other slight anomalies remain. Since little difference had been found between phosphate buffer and M-sucrose + phosphate buffer as collecting fluids at low RH for *Escherichia coli* B and commune, then if an aerosol stored at high RH were changed to low RH before collection, it might be expected that the collection difference between phosphate buffer and M-sucrose + phosphate buffer observed at high RH might disappear, but in fact it did not. *Escherichia coli* Jepp at low RH had shown poorer survival for collection in M-sucrose + phosphate buffer than in phosphate buffer, and the converse was true at high RH (Cox, 1966). Hence, if an aerosol were stored at low RH, then transferred to RH 100 % before collection it might be expected that M-sucrose + phosphate buffer would be a better collecting fluid than phosphate buffer alone. However, it was not. The collection difference would therefore not seem to be caused simply by water activity differences. Dramatic effects, both of a beneficial and of a detrimental nature, were reported by Cox (1965) and by Silver (1965) for shifts in RH to 100 % before collection.

Provided that raffinose located outside the cell wall is used to protect *Escherichia coli* B and commune and that the necessary collecting fluid is used together with an RH change to either 100 % or 30 %, depending upon the conditions, then death at or following collection can be eliminated and good survival can be achieved over the range RH 20–100 %. For *E. coli* Jepp these procedures are not sufficient to overcome the instability in the region of RH 82 %.

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REFERENCES

- Cox, C. S. (1965). The mode of action of protecting agents. *1st Int. Symp. on Aerobiology*, p. 345. Published by Naval Biological Laboratory, Naval Supply Center, Oakland, California.
- Cox, C. S. (1966). The survival of *Escherichia coli* atomized into air and into nitrogen from distilled water and from solutions of protecting agents, as a function of relative humidity. *J. gen. Microbiol.* **43**, 383.

- COX, C. S. & BALDWIN, F. (1966). The use of phage to study causes of loss of viability of *Escherichia coli* in aerosols. *J. gen. Microbiol.* **44**, 15.
- MAY, K. R. & HARPER, G. J. (1957). The efficiency of various liquid impinger samplers in bacterial aerosols. *Br. J. indust. Med.* **14**, 15.
- SILVER, I. H. (1965). Viability of micro-organisms using a suspended droplet technique. *1st Int. Symp. on Aerobiology*, p. 319. Published by Naval Biological Laboratory, Naval Supply Centre, Oakland, California.