The Aerosol Survival of *Escherichia coli* JEPP Sprayed from Protecting Agents into Nitrogen Atmospheres under Changing Relative Humidity Conditions

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(Accepted for publication 19 April 1967)

SUMMARY

The aerosol survival of *Escherichia coli* JEPP, a particularly sensitive strain, has been studied in a nitrogen atmosphere (> 99.9%) when sprayed from suspension in solutions of sodium glutamate or glycerol, and collected into phosphate buffer with or without M-sucrose. The response caused by changes in relative humidity (RH) to 30% and 100% before collection was also examined. These two protecting agents were selected because glycerol readily permeated the cytoplasmic membrane, whereas sodium glutamate did not. *E. coli* JEPP sprayed as a suspension in sodium glutamate and collected in phosphate buffer showed two minima in the survival *versus* RH curves: (i) a comparatively narrow zone between 90 and 82% RH; (ii) a broad instability zone between 82 and 50% RH. The presence of sucrose in the collecting fluid increased the survival for storage above 50% RH, but decreased it below 50% RH. For storage above 50% RH a change to 100% RH before collection increased the survival for collection in phosphate buffer but not in phosphate buffer+sucrose; below 50% RH this change decreased the survival. A change to 30% RH before collection had little effect. In similar experiments with glycerol a gradual decline in survival occurred as the RH value was decreased and no critical minima were apparent. Addition of sucrose to the phosphate buffer collecting fluid increased survival. A similar improvement was obtained by changing the RH to 100% before collection into phosphate buffer. The combined effect of having sucrose in the collecting fluid and an RH change were not additive, and were somewhat antagonistic for aerosols stored at low RH values. A change to 30% RH before collection decreased the survival. These results are compared with those for *E. coli* JEPP sprayed from suspension in distilled water and in solutions of raffinose, and show that at high RH values glycerol provided the best protection. At low RH values glycerol was inferior to sodium glutamate and raffinose, which were similar. The importance of the manner in which water re-enters the bacterium on collection is discussed.

INTRODUCTION

The aerosol survival of *Escherichia coli* has been shown to be dependent upon a number of factors (Cox, 1966*a, b*); these include an air stress at low relative humidity (RH) caused by the toxic action of oxygen. Hess (1965) found a similar result for *Serratia marcescens*. At high RH values, regions were found where *E. coli* survival

was particularly sensitive to RH, in a manner that was dependent upon the spray and collecting fluids. Relative humidity changes to 100 or 30% immediately before collection also influenced the degree of survival, either beneficially or detrimentally, depending upon the strain of *E. coli* used and the spray and collecting fluids. The present paper reports the survival of *E. coli* JEPP, a particularly sensitive strain, when sprayed from suspension in solutions of sodium glutamate and of glycerol. Previous studies have shown that raffinose, which is a good protective agent for *E. coli* B and *E. coli* *commune*, was not particularly good for the JEPP strain stored at high RH (Cox, 1966*a, b*). Sodium glutamate and glycerol were chosen for comparison with raffinose since their permeabilities are such that it is possible to obtain different distributions within the bacterial system. Raffinose could be confined to outside the cell wall, sodium glutamate permeated the cell wall but perhaps not the cytoplasmic membrane, and glycerol permeated both the cell wall and the cytoplasmic membrane. The importance of the distribution of the protective agent was discussed previously (Cox, 1965).

METHODS

These techniques were as previously reported (Cox, 1966*a, b*), except that instead of spraying the suspension of bacteria immediately upon the addition of the protective agent to the suspension of bacteria in distilled water, the sodium glutamate was allowed to equilibrate with the bacteria at 26.5° for 1 hr and the glycerol for 30 min. before spraying.

RESULTS

Tests with sodium glutamate

Figure 1 shows the survival of *Escherichia coli* JEPP sprayed from 0.13 M-sodium glutamate in distilled water into nitrogen at an aerosol age of 25 min. for collection by raised impinger (May & Harper, 1957) into phosphate buffer with and without M-sucrose. For collection in phosphate buffer two minima were apparent between 90% and 50% RH. Beyond these regions, survival similar to that for raffinose-protected bacteria (Cox, 1966*a*) was achieved. Addition of sucrose to the phosphate buffer collecting fluid tended to smooth out the marked instability between 90% and 50% RH, but below 50% RH the sucrose collecting fluid was inferior to phosphate buffer alone. This effect of the collecting fluid was similar to that found for *E. coli* JEPP sprayed from distilled water and from raffinose solution (Cox, 1966*a*). Figure 2 gives the result when the aerosol was changed from its storage RH to 100% RH immediately before collection. Above 50% RH this treatment increased the survival for collection in phosphate buffer, but generally decreased it for collection in phosphate buffer + sucrose, with the result that the collection difference shown in Fig. 1 was decreased. Below 50% this change in RH decreased the survival for collection in both fluids. A change to 30% RH immediately before collection gave results that were generally similar to those of Fig. 1, except that for storage above 82% RH this change in RH caused a decrease of survival for collection in both fluids. Table 1 presents the results obtained for different equilibration times before spraying and shows the dramatic nature of the effect. Plasmolysis data indicated that the bacteria became plasmolysed within 5 min. following the addition of sodium glutamate (final concentration 0.5 M) to a suspension of bacteria in distilled water. Hence the influence of equilibration

time was not simply that due to sodium glutamate penetrating the cell wall. The bacteria were not observed to undergo plasmolysis ('deplasmolysis').

Tests with glycerol

Figure 3 gives the survival of *Escherichia coli* JEPP sprayed from 0.3 M-glycerol in distilled water into nitrogen at an aerosol age of 25 min. collected by raised impinger (May & Harper, 1957). The data show greatest survival at high RH with a gradual decrease in survival as the RH was lowered. Unlike raffinose (Cox, 1966*a, b*) and sodium glutamate, no critical minima were apparent. Collection in phosphate buffer was inferior to that when sucrose was added over the range RH 100–30%, unlike

Fig. 1

Fig. 2

Fig. 1. Aerosol survival of *Escherichia coli* JEPP in nitrogen, sprayed from 0.13 M-sodium glutamate, at an aerosol age of 25 min. at the storage relative humidity and 26.5°. ●, Collection in phosphate buffer; ○, collection in phosphate buffer + sucrose (M).

Fig. 2. Aerosol survival of *Escherichia coli* JEPP in nitrogen, sprayed from 0.13 M-sodium glutamate, at an aerosol age of 25 min. at the storage relative humidity and 26.5° followed by a change to 100% relative humidity prior to collection. ●, Collection in phosphate buffer; ○, collection in phosphate buffer + sucrose (M).

raffinose and distilled water (Cox, 1966*a*) and sodium glutamate. At high and intermediate RH values glycerol and sodium glutamate showed similar protecting abilities for collection in phosphate buffer + sucrose. At low RH glycerol-protected bacteria gave decreased survival as compared to those sprayed from suspension in distilled water and raffinose solution (Cox, 1966*a*) and in sodium glutamate. In Fig. 4 results are given for a change from the storage RH to 100% RH immediately before collection. Over the range 100–30% RH, this change brought about increased survival for collection in phosphate buffer; there was little effect for collection in phosphate buffer + sucrose for storage above 65% RH, but below this RH value, the RH

change decreased survival. As a consequence of this treatment together with collection in phosphate buffer, glycerol was superior to sodium glutamate as a protective agent above 60% RH, and was superior to raffinose above 70% RH. Below this RH region, even with the RH changed to 100% before collection, glycerol was inferior to distilled water, raffinose (Cox, 1966*a*) and sodium glutamate. A change to 30% RH before collection decreased the survival as compared with that of control bacteria over the range RH 100–30%; addition of sucrose to the phosphate collecting fluid again increased survival.

Table 1. *The influence of equilibration time with 0.13 M-sodium glutamate on the aerosol survival of Escherichia coli JEPP stored in nitrogen at 82% RH and 26.5°: aerosol age 25 min.*

Equilibration time (min.)	Collecting fluid	Survival (%)		
		Storage RH	100 % RH*	30 % RH*
0	PB†	0.82	8.0	1.7
	PBS†	25	6.8	0.15
30	PB	7.3	34	34
	PBS	57	45	28
60	PB	91	90	60
	PBS	83	95	100

* Aerosol stored at 82% RH and shifted to 100 or 30% RH prior to collection.

† PB = phosphate buffer; PBS = phosphate buffer + sucrose (M).

Fig. 3

Fig. 4

Fig. 3. Aerosol survival of *Escherichia coli* JEPP in nitrogen, sprayed from 0.3 M-glycerol at an aerosol age of 25 min. at the storage relative humidity and 26.5°. ●, Collection in phosphate buffer; ○, collection in phosphate buffer + sucrose (M)

Fig. 4. Aerosol survival of *Escherichia coli* JEPP in nitrogen, sprayed from 0.3 M-glycerol, at an aerosol age of 25 min. at the storage relative humidity and 26.5° followed by a change to 100% relative humidity prior to collection. ●, Collection in phosphate buffer; ○, collection in phosphate buffer + sucrose (M).

The results of Table 2 indicate that increasing the concentration of glycerol in the spray fluid from 0.3 to 1 M did not improve its protective action. Replacing phosphate buffer + sucrose by 2 or 4 M-glycerol in phosphate buffer did not result in higher survival of the aerosol sample.

Table 2. *The effect of spray fluid and collecting fluid on the 25 min. aerosol survival of Escherichia coli JEPP in nitrogen at 26.5°.*

RH (%)	Spray fluid	Collecting fluids			
		A	B	C	D
		Survival (%)			
90	Distilled water	0.82	2.3	—	—
	0.3 M-glycerol	25	75	—	—
	1 M-glycerol	29	76	72	24
80	Distilled water	0.5	1.5	—	—
	0.3 M-glycerol	24	58	—	—
	1 M-glycerol	11	33	25	37
70	Distilled water	17	2.6	—	—
	0.3 M-glycerol	6.4	20	—	—
	1 M-glycerol	7.5	28	15	28
40	Distilled water	50	7.4	—	—
	0.3 M-glycerol	1.2	11	—	—
	1 M-glycerol	1.0	6.3	3.1	5.1

Collecting fluids: A, phosphate buffer, B, phosphate buffer + M-sucrose; C, phosphate buffer + 2 M-glycerol; D, phosphate buffer + 4 M-glycerol.

DISCUSSION

Cox (1966*a*) showed that the survival of *Escherichia coli* B, *E. coli* JEPP and *E. coli* *commune*, when sprayed from suspension in distilled water or raffinose solution into nitrogen, was less at high RH than at low RH, *E. coli* JEPP being particularly unstable at high RH. The poorer survival at high RH was expressed as RH zones in which *E. coli* was especially unstable. The presence of raffinose in the spray fluid increased the survival values without completely eliminating the critical RH zones. This instability was shown, by the use of different collecting fluids or RH changes from the storage RH to 100% or 30% RH immediately before collection, to be caused by loss of viability during or following collection (Cox, 1966*a, b*). However, unlike *E. coli* B and *E. coli commune*, strain JEPP remained unstable in the region of 82% RH (Cox, 1966*b*). The present results show that a protective agent having a permeability different from that of raffinose, modified the response of survival to RH. Glycerol, being able to penetrate the cytoplasmic membrane, was the best protective agent at high RH, but at low RH was inferior to distilled water, and in this sense could be considered to be toxic. However, when compared with the survival of *E. coli* JEPP sprayed from distilled water into air (Cox, 1966*a*), glycerol would be said to have a slight protective action. This apparent anomaly is because two different phenomena are occurring: in air, oxygen is toxic at low RH for *E. coli* (to be published) and glycerol protects to some degree against this stress.

Good survival at high RH can be obtained by addition of glycerol to the spray fluid, together with a shift in RH value to 100% before collection, while at low RH either raffinose (Cox, 1966*a, b*) or sodium glutamate are better protective agents than glycerol. Since protection of *E. coli* JEPP at high RH appears to require an agent able to permeate the cytoplasmic membrane, components of the cytoplasm seem to be implicated in decreased survival at high RH value. At low RH an agent such as

raffinose, which does not penetrate the cell wall, protects (Cox, 1966*a, b*). Hence different lethal mechanisms seem to be operating at high and low RH for *E. coli* JEPP stored in nitrogen; however, these mechanisms must be influenced by the manner in which water re-enters the bacteria following collection as shown by the effects of the collection procedures. *E. coli* B can also die through different lethal mechanisms at high and low RH, as shown by Cox & Baldwin (1966). Like *E. coli* JEPP, *E. coli* B and *E. coli commune* survival can also depend upon the way in which water re-enters the bacteria during collection (Cox, 1966*a, b*). This feature of collection is not confined to aerosol droplets (about 1 μ diam.) but also applies to comparatively large droplets (about 100 μ diam.) supported upon fine glass fibres (Cox, 1965; Silver, 1965).

The author thanks Mr I. H. Silver for his interest and advice and thanks Mr C. M. Saunders for technical assistance.

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