The Aerosol Survival and Cause of Death of *Escherichia coli* K12

By C. S. COX

Microbiological Research Establishment, Porton Down, near Salisbury, Wilts.

(Accepted for publication 18 June 1968)

SUMMARY

The survival of *Escherichia coli* K12 HfrC sprayed from distilled water into a nitrogen atmosphere as a function of aerosol age and of storage relative humidity (RH) is demonstrated. The survival pattern was typically that of *E. coli*, i.e. marked instability in a region at high RH and better stability at low RH. The results of changing the RH from the storage RH to 100% or 30% are described. Comparison of survival in nitrogen with that in air showed air to be slightly toxic, the toxic component being oxygen or a trace of some contaminant in it. Glycerol and raffinose were slightly protective as additives at high RH; at low RH glycerol was toxic, but raffinose was highly protective. It was discovered that *E. coli* K12 HfrC carried a temperate phage and that this phage was not activated by the processes involved in aerosol experiments. The synthesis of phages T3 and μ 2 by *E. coli* K12 HfrC, collected from the aerosol, was examined. Phage production and viability were similar, and hence it is concluded that in a nitrogen atmosphere loss of viability was not caused by DNA inactivation, DNA synthesis inhibition or inhibition of cell-wall division, but by failure of RNA synthesis, protein synthesis or energy production.

INTRODUCTION

Microbial survival in aerosols was reviewed in general terms by Anderson & Cox (1967). In particular, the aerosol survival of *Escherichia coli* has been found to depend upon several variables (Cox, 1965, 1966*a*, *b*, 1967, 1968; Cox & Baldwin, 1964, 1966, 1967). These include an 'air stress' (Cox 1966*a*) at low relative humidity (RH), caused by the toxic action of oxygen, possibly modified by the presence of contaminants (Cox & Baldwin, 1967; Hess, 1965; Webb, 1967). At high RH, regions were found where *E. coli* survival was particularly sensitive to RH, in a manner dependent on the spray fluid and collecting fluid (Cox, 1966*a*, *b*, 1967, 1968; Cox & Baldwin, 1966). Relative humidity changes imposed before collection of the aerosol also influenced survival, either beneficially or detrimentally, depending upon the strain of *E. coli* used and the nature of the spray and collecting fluids (Cox, 1966*b*, 1967). Such results showed that the death mechanisms of *E. coli* in nitrogen atmospheres must be influenced by the manner in which water re-enters the bacteria during collection, e.g. when *E. coli* B and COMMUNE (M.R.E. no. 162) are sprayed from raffinose into nitrogen at high RH (Cox, 1966*a*, *b*). Furthermore, it was shown that at high RH (where oxygen is not toxic, Cox & Baldwin, 1967) atmospheres of nitrogen, argon and helium were not completely biologically inert (Cox, 1968). Analysis of these results also indicated that the initial evaporation rates of the aerosol droplets did not influence the long-term survival of *E. coli* B in the aerosol. As a consequence it was thought likely that

RH was important with regard to survival through its effect on the water content of bacteria in the aerosol; RH and water content are related through the water sorption isotherm. Cox (1968) suggested that protective agents do not operate through a modification of the initial evaporation rate of aerosol droplets.

The present paper reports the aerosol survival as a function of RH of *Escherichia coli* K12 HfrC sprayed into air and into nitrogen. The object of the work was: first, to determine whether *E. coli* K12 HfrC behaved like other strains of *E. coli*, and, secondly, as it is a male-specific bacterium, to examine the ability of *E. coli* K12 HfrC collected from the aerosol to reproduce phage μ 2 (RNA) and phage T3 (DNA). This approach has been previously reported for *E. coli* B and phage T7 (Cox, 1965; Cox & Baldwin, 1964, 1966; Webb, Dumasia & Singh Bhorjee, 1965).

METHODS

The techniques used were as previously reported (Cox, 1966*a*, *b*, 1967). *Bacillus subtilis* var. *niger* was used as a tracer (Anderson & Cox, 1967). At very high RH *B. subtilis* var. *niger* spores lose viability in the aerosol (Cox, 1966*a*). However, as discussed by Cox (1968), in order to obtain fine control of RH it is necessary to use a single-jet spray with, consequently, a low output. The only tracer method which is sufficiently sensitive is the spore tracer (Anderson & Cox, 1967). Aerosol samples for assay of phage μ 2 and phage T3 synthesis were collected in liver-digest broth + anti-foam in place of the more usual phosphate buffer or phosphate buffer + sucrose (Cox, 1966*a*). Samples (2 ml.) of aerosol were warmed to 37°, and suitably diluted. Phage μ 2 and phage T3 were added to respective samples to give a phage: bacterium ration of 3:1. As a control, the spray fluid was diluted to give the same number of coli organisms/ml. as in the aerosol samples, and was then treated similarly to the aerosol samples. Following incubation for 2 hr. at 37° serial dilutions were made and 1 ml. was added to 2 ml. blood agar base (0.7% agar, tryptone, liver digest, yeast extract, sodium chloride) at 45°. The diluted agar was seeded with *Escherichia coli* K12 HfrC and poured on Petri dishes containing blood agar base (1.5% agar). After incubation for 18 hr. at 37° the plaques were counted. Six plates/dilution were used.

Previous studies (Cox & Baldwin, 1964, 1966) of the ability of *Escherichia coli* B to reproduce phage T7 were made by direct observation of *E. coli* B with a microscope. This technique was found to be unsuitable for *E. coli* K12 HfrC because lysis was not as definite as with *E. coli* B.

RESULTS

Escherichia coli K12 HfrC sprayed from distilled water

Figures 1 to 3 give the survival in nitrogen at 0.3 sec., 2 min., 15 min. and 30 min. collected into phosphate buffer. Figure 3 also gives the result for a collecting fluid of phosphate buffer + M-sucrose and indicates a similar phenomenon to *Escherichia coli* JEPP, namely, that at low RH the addition of sucrose to the collecting fluid decreased survival. The survival pattern was typically that for *E. coli*, namely, a region at high RH where *E. coli* was particularly unstable, while at low RH greater survival was obtained. The unstable region at high RH was particularly marked at an aerosol age of 2 min., where at 85% RH the survival was 0.13%. As the aerosol aged the minimum widened to give 0% survival at RH 85–70% inclusive. Since the minimum was less

critical with regard to RH than was found with *E. coli* B, JEPP and COMMUNE (M.R.E. no. 162), this K12 strain of *E. coli* was ideally suited to a study of the mechanism which caused such a minimum.

Fig. 1. The aerosol survival of *Escherichia coli* K12 HfrC sprayed from distilled water into nitrogen at 26.5° as a function of relative humidity. ●, 0.3 sec. aerosol age; ×, 2 min. aerosol age. Phosphate buffer collecting fluid.

Fig. 2. The aerosol survival of *Escherichia coli* K12 HfrC sprayed from distilled water into nitrogen at 26.5° as a function of relative humidity, at an aerosol age of 15 min. Phosphate buffer collecting fluid.

Figure 4 gives the results for RH changes from the storage RH to either 100% or 30% prior to collection of the aerosol. A change to 100% RH caused little improvement in survival at high RH and lowered survival at low RH. Conversely, a change to 30% RH lowered survival at high RH and at low RH had little effect. These results indicate that the rehydration process during collection can be very important with regard to survival.

Table 1 compares the survival at 30 min. aerosol age for *Escherichia coli* K12 HfrC sprayed from distilled water into air and into nitrogen. It is evident that air was slightly toxic, and by using mixtures of nitrogen and oxygen the lower survival in air was shown to be caused by oxygen, or a trace contaminant in it (see also Cox & Baldwin, 1967).

Fig. 3

Fig. 4

Fig. 3. The aerosol survival of *Escherichia coli* K12 HfrC sprayed from distilled water into nitrogen at 26.5° as a function of relative humidity, at an aerosol age of 30 min. ●, Phosphate buffer collecting fluid; x, phosphate buffer + M-sucrose collecting fluid.

Fig. 4. The aerosol survival of *Escherichia coli* K12 HfrC sprayed from distilled water into nitrogen at 26.5° as a function of relative humidity, at an aerosol age of 31.5 min. ●, Relative humidity changed to 100% before collection; x, relative humidity changed to 30% before collection. Phosphate buffer collecting fluid.

Table 1. The survival of *Escherichia coli* K12 HfrC sprayed from distilled water at an aerosol age of 30 min., as a function of relative humidity

Collecting fluid was phosphate buffer. Temperature 26.5°.

Relative humidity (%)	95	85	75	65	55	45	35	25	20
Survival in air (%)	1.2	0	0	0	4.4	7.0	10	3.0	7.2
Survival in nitrogen (%)	4.5	0	0	0.64	7.8	15	16	16	12

Escherichia coli K12 sprayed with protective agents

Previous studies (e.g. Cox, 1967) investigated the protective action of glycerol and of raffinose. These substances were chosen because glycerol readily permeated *Escherichia coli*, whereas raffinose only very slowly penetrated the cell wall. Their effects on *E. coli* K12 HfrC are given in Table 2, together with the survival for *E. coli* K12 HfrC sprayed from distilled water. The data show that glycerol was slightly protective at high RH and toxic at low RH; raffinose showed slight protection at high RH and good

protection at low RH. This pattern has been found with other strains of *E. coli* (e.g. Cox 1967).

Table 2. The survival of *Escherichia coli* K12 HfrC sprayed from protective agents as a function of aerosol age and of storage relative humidity

Collecting fluid was phosphate buffer, except: (*) which was phosphate buffer + M-sucrose; (†) RH changed to 100% prior to collection; (‡) RH changed to 30% prior to collection. Glycerol was equilibrated with the bacteria for 15 min. before spraying. Raffinose was added immediately before spraying. Temperature 26.5°.

Spray fluid RH (%)	% survival											
	Glycerol, 0.3 M				Raffinose, 0.15 M				Distilled water			
	85	75	45	25	85	75	45	25	85	75	45	25
Aerosol age												
0.3 sec.	98	63	26	38	93	86	85	85	80	73	38	22
2 min.	15	3.2	0	0.9	15	48	81	92	0.13	4.5	31	26
15 min.	6.4	1.3	0	4.4	0	16	75	79	0	0	9.4	19
30 min.	3.6	0	0	3.1	1.0	4.8	69	84	0	0	7.9	17
30 min.*	17	2.2	0	6.6	4.9	5.5	34	28	0.58	0	0.41	8.4
31.5 min.†	17	0	0	0	0	3.4	39	38	0	0	0.58	1.6
31.5 min.‡	1.7	0	0	0	0	3.4	73	79	0	0	14	20

Table 3. The synthesis of phage T3 and phage μ 2 by *Escherichia coli* K12 HfrC collected from the aerosol

Aerosol age 30 min.; temperature of storage 26.5°. Collecting fluid liver-digest broth + anti-foam.

RH of aerosol (%)	Phage T3 production (%)	Phage μ 2 production (%)	Viability (%)
85	0	0	0.5
30	22	9	14

The temperate phage of *Escherichia coli* K12 HfrC

It was suspected that *Escherichia coli* K12 HfrC might carry a temperate phage. Ultraviolet irradiation or heating at 50° for 30 min. activated the temperate phage, which readily plaqued on *E. coli* K12 HfrC and *E. coli* B, suggesting that it was a DNA phage. This situation gave rise to another possible death mechanism for *E. coli* K12 HfrC, namely that aerosolization activated the temperate phage, which then directed the metabolism of *E. coli* K12 HfrC and prevented colony formation. But, although activation of the temperate phage was looked for at high, intermediate and low RH, none was found. Hence this death mechanism did not occur for *E. coli* K12 HfrC, but may occur with other bacteria and should not be overlooked.

The synthesis of phage T3 and phage μ 2 by *Escherichia coli* K12 HfrC collected from the aerosol

Table 3 gives the % phage production and % viability. The data show that at 85% RH neither phage T3 nor phage μ 2 was produced, while at 30% RH phage production was similar to the extent of colony formation. Therefore, death of *Escherichia coli* K12 HfrC can be attributed to the failure of a metabolic function which was common to the multiplication of *E. coli* K12 HfrC and to the production by this bacterium of phages T3 and μ 2.

DISCUSSION

In general, the survival of *Escherichia coli* K12 HfrC followed the pattern for *E. coli* B JEPP and COMMUNE (M.R.E. no. 162). Of the four coli strains studied, *E. coli* K12 HfrC was the most unstable at high and at low RH, and was a good organism with which to study death mechanisms other than oxygen toxicity (Cox & Baldwin, 1967), because of the extent of viability loss when sprayed from distilled water into a nitrogen atmosphere. The minimum at 85% RH was very similar to that found at the same RH for *E. coli* B, but was very apparent at a much shorter aerosol age. The effect of the collecting fluid was also similar to that for *E. coli* JEPP (Cox, 1966*a*, *b*, 1967). This effect, and that caused by RH changes before collection, indicated, as for the other three strains of *E. coli* studied, that the manner in which water enters the bacterium during collection influences the survival. Oxygen toxicity was less apparent with *E. coli* K12 HfrC than, for example, *E. coli* B (Cox, 1966*a*; Cox & Baldwin, 1967), probably owing to the poor survival of the former in nitrogen at low RH (in the absence of protective agents). The action of glycerol and raffinose was very similar to that with *E. coli* JEPP (Cox, 1967), and again showed that at low RH a protective agent (namely raffinose) outside the cell wall was able to confer stability.

The fact that *Escherichia coli* K12 HfrC carried a temperate phage suggested an interesting death mechanism, although in this case it was shown to be inoperative. Hayes (1964) reported that *E. coli* B carried a prophage, but its activation as a cause of death of *E. coli* B can probably be excluded since Cox & Baldwin (1966), who observed *E. coli* B collected from the aerosol onto a nutrient agar by means of a microscope, found that *E. coli* B did not lyse. However, it is possible that the *E. coli* B phage was activated and reproduced without lysis of the *E. coli* B. The results obtained for phage T3 and phage μ 2 production by *E. coli* K12 HfrC collected from the aerosol strongly suggest that neither DNA inactivation, DNA synthesis inhibition nor inhibition of cell-wall division were the cause of the loss of viability of *E. coli* K12 HfrC in nitrogen. Failure of these processes would not prevent phage production, especially as phage μ 2 is an RNA phage which can replicate independently of DNA metabolism. It is therefore possible to conclude that the cause of death of *E. coli* K12 HfrC as an aerosol in nitrogen was because of failure of RNA synthesis, protein synthesis or energy production. The finding that DNA metabolism was not involved in the death mechanism is in agreement with the results of Benbough (1967), who used an entirely different technique. Since DNA inactivation is unlikely, then the results of Cox & Baldwin (1966) can be interpreted to suggest strongly that the toxic action of oxygen is to interfere with cell-wall synthesis and cell division.

The author thanks Mr C. M. Saunders for technical assistance.

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