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Survival of *Escherichia coli* with and without ColE1::Tn*5* after Aerosol Dispersal in a Laboratory and a Farm Environment

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The survival of a laboratory strain and a naturally occurring fecal strain of *Escherichia coli*, with and without a Tn*5*-containing derivative of ColE1, was examined after aerosol dispersal in a laboratory office and a barn under ambient temperature and humidity conditions. Following the release of paired strains, air and diverse types of surfaces were assayed for the test organisms. In both environments, the number of airborne bacteria declined rapidly within the first 2 h. Longer survival was found on surfaces and varied with surface type: recovery was greatest from wood products. Organisms persisted for 1 day in the office and for up to 20 days in the barn. Survival of the fecal strain was better than that of the laboratory strain in both test environments. In general, plasmid-bearing strains fared similarly to their plasmidless parents, but in several comparisons the ColE1::Tn*5*-containing strain showed enhanced survival. These studies have implications for the present and proposed release of genetically engineered organisms with and without plasmid vectors.

There is growing interest in using genetically engineered microorganisms to perform beneficial ecologic functions. Aerosol dispersal is one means of applying these organisms. However, data relevant to the survival of host-vector systems after their deliberate or accidental release as aerosols into the environment are limited. Prior studies have focused on aspects of bacterial survival within closed environmental ecosystems under closely controlled laboratory conditions (4–6, 13; D. L. Hunt, Ph.D. thesis, University of North Carolina, Chapel Hill, 1984). Most of these were short-term studies performed primarily to determine factors contributing to the death of bacterial aerosols. We sought to examine and compare the long-term survival of laboratory and naturally derived *Escherichia coli* strains (with and without a plasmid vector) in environments more closely representing those which would be involved in the purposeful or accidental release of genetically engineered organisms. A laboratory office and a farm were chosen to represent two diverse environments for study, and two biochemically marked *E. coli* strains, one laboratory derived and one isolated from human feces, were selected for dissemination. We studied the fate of released organisms in air and on a wide range of components of the test ecosystems. Because of current governmental restrictions on the release of genetically engineered plasmids, we used the parent cloning vector ColE1 and introduced the kanamycin resistance transposon Tn*5* by natural processes. The two *E. coli* strains, with and without ColE1::Tn*5*, were paired in different combinations and released as aerosols under conditions of ambient temperature and relative humidity.

To our knowledge, this is the first reported study in which bacterial survival has been followed after deliberate aerosol release into an uncontrolled natural environment. The testing of bacteria in pairs permitted the comparison of two different bacteria under the same environmental conditions.

MATERIALS AND METHODS

Test environments. Two natural but different environments were selected as sites for the release of the test bacteria. The choices were designed to represent one "indoor" site, a space typical of an environment where genetically engineered bacteria are generated and handled in large quantities, and one "outdoor" site, a barn situated on a private farm.

The indoor site consisted of a 1,500-ft^3 (ca. 42.48-m^3) office (12.5 by 15 by 8 ft) located within a 1,000-ft^2 (ca. 92.9-m^2) microbiology laboratory. To maintain the bacteria in the environment, we shut down the ventilation and air conditioning systems and kept the windows closed. The door was closed during the entire 10 min of aerosol release but was opened intermittently thereafter for 5-s periods to permit entry and exit of personnel who were taking test samples. Fluorescent lights were left on during the day and turned off at night. No attempt was made to control the temperature or relative humidity, but these variables were recorded throughout each experiment. Aerosols were generated from the middle of the room, and test sites were selected at various heights and distances from the aerosol-producing apparatus. The outdoor test site was the semienclosed environment of a barn situated on a small private farm in Sherborn, Mass., 25 mi (ca. 40.23 km) west of Boston. To better evaluate a large number of testing sites and surfaces, we selected an enclosed room measuring 9 by 11.5 by 8.5 ft (880 ft^3) (ca. 24.91 m^3) located in the corner of the barn (195 by 24 ft [ca. 59.44 by 7.32 m]). The room had previously housed young calves and contained used animal bedding consisting of hay and wood shavings. No attempt was made to clean the room. The floor was frequently wet because a water-feeding hose for the animals originated from the center of the floor. The door and windors were kept closed during bacterial release, but the door was opened intermittently for passage of the researchers. Aerosols were subjected to

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natural lighting conditions as well as ambient temperature and relative humidity, which were recorded daily. Bacteria were dispersed from the middle of the room, and test areas and surfaces were selected at known heights and distances from the dispersal site. Flies, wood chips, animal bedding, water, and muck (consisting of wet dirt, animal bedding, and rodent feces) were sampled (up to 50 g) when available.

Bacterial strains and plasmids. Two strains of *E. coli* were used: a laboratory K-12 strain, χ1666 (Nal^r Ara^-) (11), and an antibiotic-susceptible wild-type strain, SLH25, isolated from the feces of a laboratory worker and identified by using an API 20E biochemical test strip (Analytab Products, Plainview, N.Y.).

Microbiologic assessments of the environmental test sites showed that bacteria with different antibiotic resistances were present; however, rifamycin resistance (Rif^r) was low to nondetectable, and media containing 50 to 100 μg of rifamycin per ml provided an effective means of selection for the test organisms. Spontaneous chromosomal mutation to Rif^r was selected in both strains by successive (stepwise) plating on agar containing up to 100 μg of rifamycin per ml.

ColE1 was selected as the plasmid of choice because it is a naturally occurring plasmid which has been used as the parent vehicle for the construction of many of the currently used engineered cloning vectors (e.g., pBR322, pBR325) (3). To provide a traceable selective marker for the ColE1 plasmid, we introduced the kanamycin resistance (Kan^r) transposon Tn*5*. ColE1 DNA was first transformed into r^- m^+ (restriction, modification) *E. coli* MM294 (*supE44 thi-1 endA1*) (obtained from A. Wright) to adapt it for introduction into wild-type *E. coli*. Subsequently, the ColE1-bearing *E. coli* MM294 host was infected with λ carrying the Tn*5* transposon. Cells bearing Tn*5* on the plasmid (as opposed to the chromosome) were enriched by selection on media containing high levels of kanamycin (100 μg/ml). The ColE1::Tn*5*-bearing colonies were subsequently confirmed by gel electrophoresis, and the ColE1::Tn*5* DNA was transformed into the *E. coli* laboratory strain HB101. The plasmid DNA was again extracted and used to transform *E. coli* χ1666 and SLH25. The presence of the plasmid was confirmed by rapid plasmid isolation techniques (10) and DNA-DNA hybridization by the Southern method (14) with a nick-translated ^{32}P-labeled ColE1 probe (10). Purified isolates of both strains were stored in soft agar stabs and reisolated on agar with 100 μg of rifamycin per ml prior to release.

Media. Initial studies comparing different collection media in the assay systems determined that MacConkey agar was 30 to 50% less efficient than L (Lennox) agar (9) with 0.5% glucose in the recovery of airborne gram-negative bacteria. L agar containing 0.5% glucose, 50 μg of rifamycin (Sigma Chemical Co., St. Louis, Mo.) per ml, and 2 μg of amphotericin B (Sigma) per ml (to inhibit molds) allowed for the efficient recovery of test bacteria and inhibited indigenous "background" flora. A disadvantage of using L agar was that insects were attracted to it, and contamination by insect vectors was evident over long-term sampling periods (≥24 h).

TABLE 1. Experimental conditions

Expt	Strain(s) (aerosol output)[a]	A/B ratio	Temp (°F)[b]			Relative humidity		
			Initial	Range		Initial	Range	
				First 5 h	Subsequent days[c]		First 5 h	Subsequent days[c]
Laboratory								
Pilot	χ1666 (4.0×10^8)[d]		72	73–77	71 (1)	24	19–23.5	36 (1)
1	A χ1666 (4.4×10^8)	0.54	79	75–80	70–78 (5)	35	34–39	22–66 (5)
	B SLH25 (8.2×10^8)							
2	A χ1666 (3.1×10^8)	1.5	70	70–72	68 (1)	15.5	9.5–13	12.5 (1)
	B χ1666(ColE1::Tn*5*) (2.1×10^8)							
3	A SLH25 (6.6×10^8)	1.9	73	73–74	71 (1)	43	33–43	39 (1)
	B SLH25(ColE1::Tn*5*) (3.4×10^8)							
4	A χ1666(ColE1::Tn*5*) (3.5×10^7)	0.10	72	72	70–72 (2)	50	50–53	42–46 (2)
	B SLH25(ColE1::Tn*5*) (3.4×10^8)							
Barn								
5	A χ1666 2.5×10^8)	0.53	60	60	56–62 (13)	82	82–94	55–99 (13)
	B SLH25 (4.7×10^8)							
6	A χ1666 (2.2×10^8)	1.1	72	68–72	67–78 (7)	70	70–72	55–75 (7)
	B χ1666(ColE1::Tn*5*) (2.1×10^8)							
7	A SLH25 (1.2×10^8)	0.46	79	77–80	75–81 (7)	64	64–70	64–82 (7)
	B SLH25(ColE1::Tn*5*) (2.6×10^8)							
8	A χ1666(ColE1::Tn*5*) (4.0×10^8)	0.25	72	72–73	65–74 (5)	61	61–68	57–80 (5)
	B SLH25(ColE1::Tn*5*) (1.6×10^9)							

[a] Number of bacteria aerosolized over a 10-min period.
[b] To convert degrees Farenheit to degrees Celsius, subtract 32, multiply by 5, and divide by 9.
[c] Numbers in parentheses represent the number of days after the first day of aerosol dispersal.
[d] Released over a 5-min period.

Subsequent to collection on L agar containing rifamycin, colonies were evaluated and then replica plated (8) to MacConkey agar containing arabinose and nalidixic acid (30 μg/ml) for the identification of χ1666. ColE1::Tn*5*-bearing colonies were identified by resistance to kanamycin (10 μg/ml).

Aerosol generation. A three-jet (MRE-type) Collison nebulizer (BGI, Inc., Waltham, Mass.) was selected to produce aerosols (1- to 10-μm particles) of the bacterial suspensions by using air compressed at 20 lb/in^2 (12). For each experiment, bacterial colonies were selected from agar containing 100 μg of rifamycin per ml, grown to the late log phase in L broth, washed twice in cold L broth (Hunt, Ph.D. thesis), resuspended in 25 ml of cold L broth, and kept on ice prior to dispersion.

The numbers of test bacteria generated in each aerosol experiment were determined by the duplicate spraying of a portion of each bacterial suspension. Using the arrangement described by Hunt (Ph.D. thesis), we immediately "captured" the aerosols in two all-glass impingers (Ace Glass Co.) each containing 20 ml of L broth with 0.01% Antifoam A. The resulting suspensions were serially diluted and plated for calculation of the aerosol output.

Four 10-min bacterial releases were performed in each environment. Initially, a 5-min pilot release of χ1666 alone was conducted in the laboratory office. All other experiments were performed in pairs which tested the survival of either (i) the wild-type versus the laboratory strain or (ii) two isogenic strains, one of which carried the ColE1::Tn*5* plasmid. The total bacteria dispersed in each experiment ranged from 3.5×10^7 to 1.6×10^9 CFU (Table 1). The experimental conditions of temperature and relative humidity were recorded during the first 5 h and daily thereafter (Table 1). Ambient temperatures during the release and initial testing periods ranged from 70 to 79°F (ca. 21.1 to 26.1°C) in the laboratory and 60 to 79°F (ca. 15.6 to 26.1°C) in the barn. The relative humidities varied considerably; those at the farm (61 to 82%) were up to fourfold higher than those in the laboratory (15.5 to 50%).

Assay systems. Three well-defined, standard methodologies were used for the recovery, enumeration, and identification of bacteria from the environment. For recovering airborne bacteria, we used the six-stage Andersen microbial air sampler (1), a portable device which draws one cubic foot (28.3 dl^3) of air per minute through a series of six cascading sieves and impacts bacteria on agar-containing plates. The device was prepared and used in accordance with manufacturer instructions. For sampling, it was placed in the center of the floor of the test environment and run for 5 s to 15 min during the first hour after spraying. Subsequently, samples were taken for 1 h for a total of 4 to 5 h and daily thereafter.

Larger particles which settled out by gravity were col-

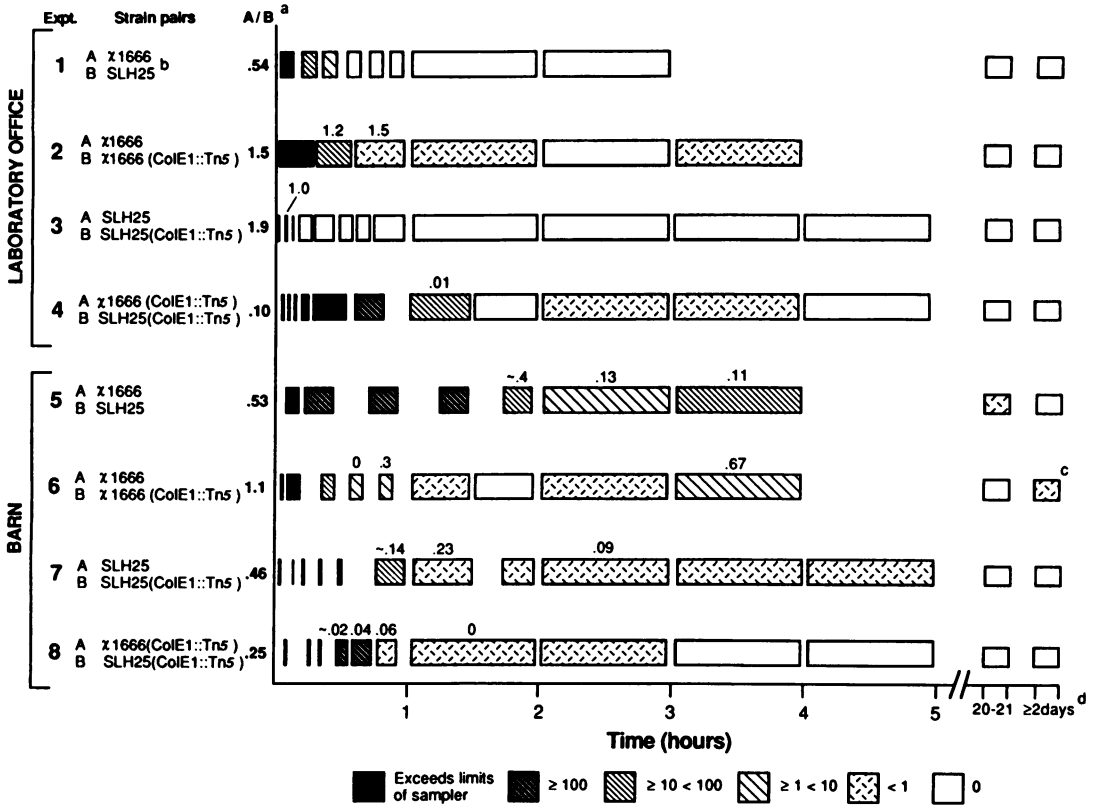

FIG. 1. Recovery of airborne bacteria via the Andersen sampler. The survival of bacteria in air was assessed after eight separate aerosol dispersals of paired strains of *E. coli* in two environments (laboratory office and barn). In the first experiment (χ1666 and SLH25 in the office), few wild-type organisms were recovered because the level of rifamycin (75 μg/ml) in all of the recovery media was too high. Bars represent total numbers of CFU/ft^3 per min as recovered on L agar containing rifamycin. In samples having 20 or more colonies, strain pairs were differentiated by using the Ara$^-$ Nal^r (for χ1666) and Kan^r (for ColE1::Tn*5*) phenotypes, and the ratio of the two (A/B) was calculated (numbers over bars). a, Initial ratio of released strains. b, No recovery of SLH25 (see text). c, Recovery of one χ1666(ColE1::Tn*5*) colony on day 5. d, Results of a 1-h test with the Andersen sampler on each day after the day of aerosol dispersal.

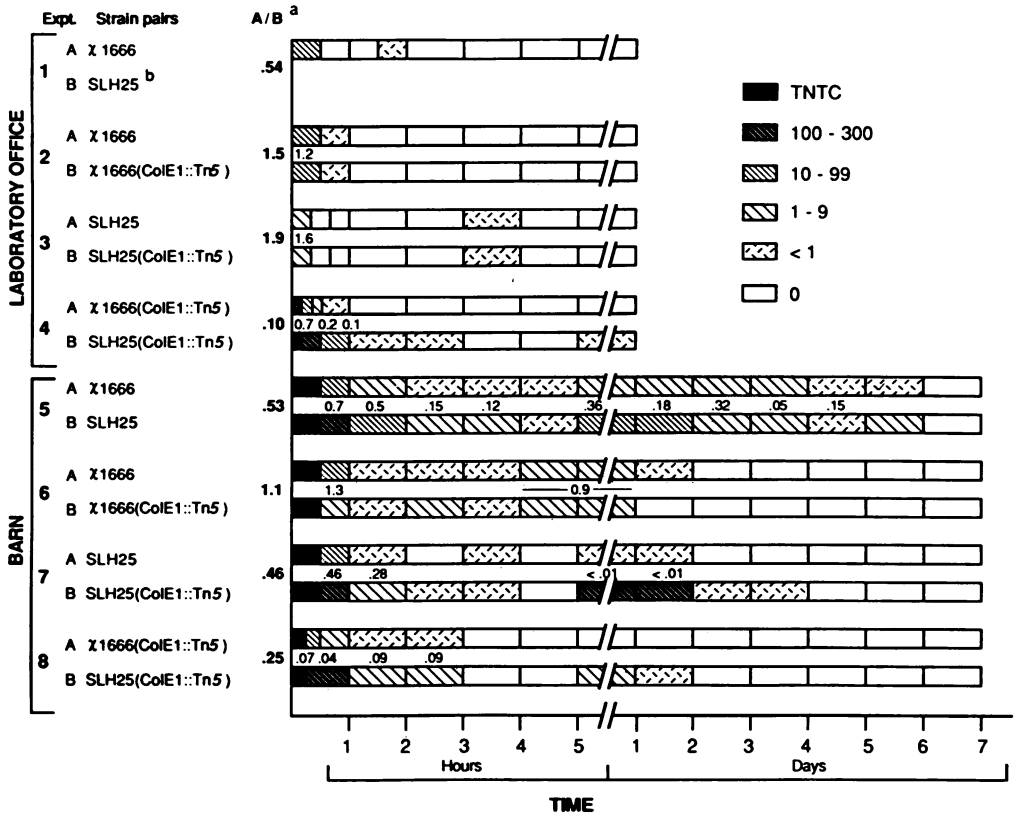

FIG. 2. Recovery of bacteria released as described in the legend to Fig. 1 from GSC. Bars represent the mean numbers of CFU per plate as recovered on L agar with rifamycin. Numbers over bars represent the A/B ratio (see Fig. 1). Plates placed in the barn after 5 h showed gross contamination by insects and rodents. TNTC, Too numerous to count. See the legend to Fig. 1 for other details. a, Initial ratio of released strains. b, No recovery of SLH25 (see text).

lected by means of open petri dishes (gravitational settling cultures) (GSC). Approximately 18 GSC plates were placed at selected distances from the Collision nebulizer immediately after the 10-min spraying period and left for periods of 10 to 60 min. Sampling was repeated for a total of 5 h with 60-min sampling periods. Subsequently, sample plates were left for periods of 24 or 48 h. The numbers of bacteria (CFU) were evaluated after overnight incubation at 37°C.

Contact plates (Nunc, Roskilde, Denmark) containing a convex surface of selective agar were used for pressing against test surfaces. Eighteen samples were taken immediately after bacterial release. Sampling was repeated (but not at the exact same spot) at selected times during the first 5 h and daily thereafter. In addition, two investigators sampled skin, hair, and clothing periodically after exiting the test rooms.

Substances such as wood chips, animal bedding, muck, and feces were quantitatively analyzed by preparing 10-fold serial dilutions (weight/volume) in sterile buffered saline with up to 50-g samples. Homogenization was performed for 60 s in a Waring blender. Samples of flies were diluted 10-fold, macerated by hand, and vortexed vigorously before being plated on selective agar. Water samples were serially diluted and plated, or alternatively, 100-ml samples were filtered by suction through an analytical filter funnel (type AF, 0.45-μm pores; Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.) and the filter was placed on selective agar.

RESULTS

Recovery of bacteria from air and surfaces. In general, large numbers of suspended bacteria were viable up through the first 30 min after release. However, in all experiments except one (Fig. 1, experiment 5), the viability dropped down to ≤1 CFU/ft^3 (28.3 dl^3) within 1 to 2 h. A few isolated CFU could be recovered for several hours, but in only two experiments (Fig. 1, experiments 5 and 6 in the barn) were any bacteria detected on subsequent days.

Similar recoveries on widely dispersed GSC plates indicated rapid dissemination of the bacteria to all parts of the test room within the first few minutes. In the office environment, fallout decreased rapidly, such that little or no bacteria could be recovered at 4 h regardless of the experimental conditions or bacterial strains involved (Fig. 2). In only one instance were CFU recovered at 24 h (Fig. 2, experiment 4). In the barn, bacteria were recovered on GSC plates for several days after spraying despite the negative recovery obtained with the Andersen sampler. In some instances, these plates actually showed increased numbers of test bacteria over time. This finding was presumably the result of either resuspension of fallout bacteria or contamination by flying and crawling insects as well as rodents which were attracted to the agar. The latter probably account for the majority of CFU recovered after day 1 in the barn, since

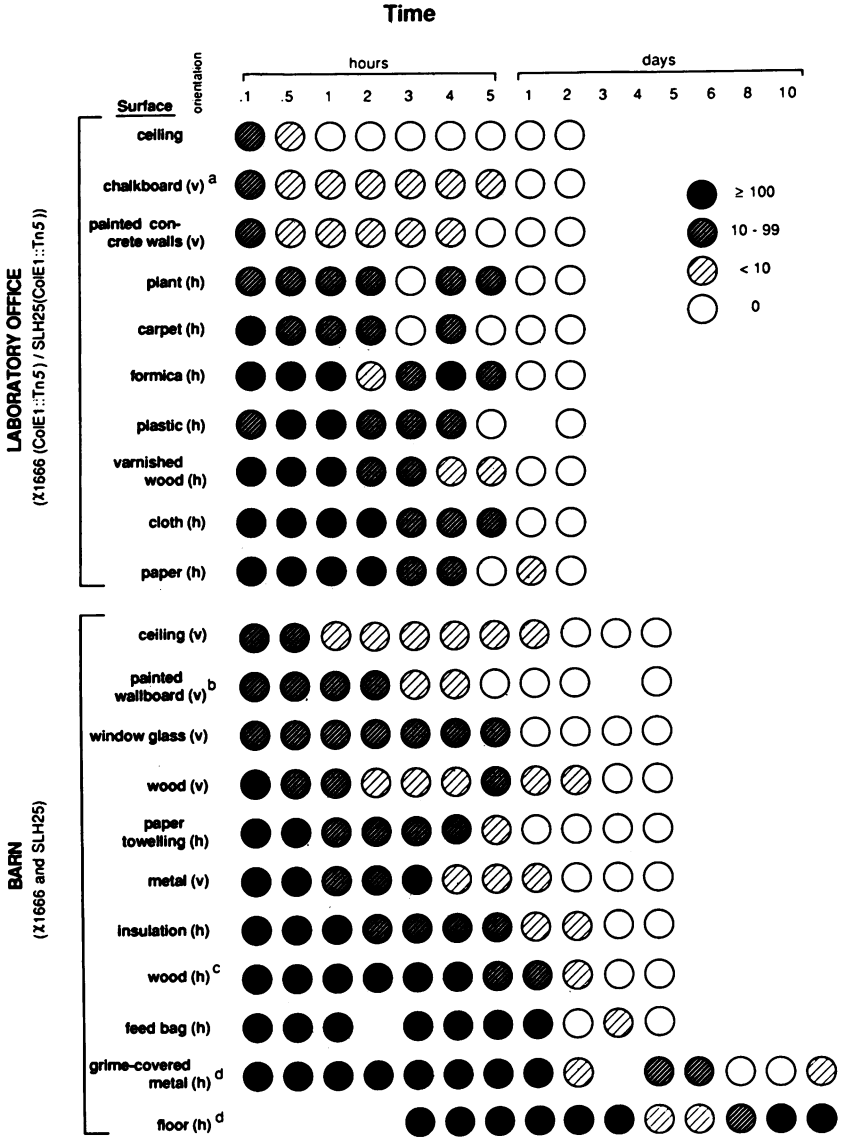

FIG. 3. Recovery of bacteria from the surfaces of different substances. One experiment from each environment (experiments 4 and 5 in Fig. 1 and 2), representing the maximum recovery from that environment, was chosen. Circles represent the numbers of total CFU per plate (or the means of total CFU) recovered from a sampling site(s) on each surface type. In both experiments, the wild-type strain survived significantly better (see Table 3). a, Spatial orientation of surface type: v, vertical; h, horizontal. b, One or two CFU (wild-type strain) were recovered on days 4 and 10; one wet surface produced 10 to 99 CFU (wild-type strain only) through day 10. c, One wet surface produced confluent growth of the wild-type strain only through day 13. d, Sample sites were negative on day 13 and thereafter.

there was evidence of insect tracks and the marks of rodent teeth.

In two of the experiments (experiments 7 and 8), we tested recovery of bacteria outside the test room immediately after release. A few CFU (mean, $\leq$50 per plate) were recovered from plates placed within a 10-ft (ca. 3.05-m) radius of the test room; however, no bacteria were recovered 60 min after release.

CFU varied widely on different surface types. One experiment from each environment in which bacteria survived the longest is presented in Fig. 3. Overall recoveries on contact plates were markedly higher in the barn than in the office. The longest survivals on surfaces in the office were obtained from ordinary paper (through 21 h). In the barn, CFU could readily be recovered for several days from a variety of surfaces, such as bare wood, insulation, and grime-covered surfaces. Bacteria persisted for a maximum of 10 days on cement and wooden flooring (Fig. 3). Extensive testing of surfaces outside the experimental barn room failed to recover any test bacteria. These included pig skin and waste, chickens and their eggs, cow feces, and numerous sites of floors and wooden walls.

The hair, skin, and clothing of two research personnel were sampled periodically with contact plates after exit from the test rooms. Organisms could be recovered from hair as long as 5 h after aerosol release and up to at least 2 h on clothing. No viable organisms were detected on skin after 70 min.

Recovery from other environmental components. Of all materials tested, wood shavings supported both strains for

TABLE 2. Recovery of test strains from wood shavings and water

Expt	Strains[a]	Material[b]	Day(s) post-aerosol dispersal	Avg titer/g	A/B ratio
5	A χ1666 (0.53)	Wood shavings	3	4.9×10^3	0.15
	B SLH25		4	1.8×10^3	0.09
			6	2.3×10^2	<0.01
			8	1.1×10^3	0.33
			10	1.9×10^2	0.42
			13	1.3×10^2	<0.01
			20	0.5×10^1	—[c]
			28	ND[d]	
		Water	0	NQ[e]	
			1	NQ	
			2	4.5×10^2	0.14
			3	5.5×10^2	0.07
			4	6.0×10^1	<0.01
			8	0.1×10^1	—
			10[f]	0.02×10^1	—
7	A SLH25 (0.46)	Wood shavings	0	5.0×10^1	0.08
	B SLH25(ColE1::Tn*5*)		1	1.3×10^3	0.15
			2	1.5×10^3	0.16
			3	3.5×10^2	—
			4	6.7×10^1	<0.01
			5	ND	
			7	1.3×10^2	—
			13	ND	
8	A χ1666(ColE1::Tn*5*) (0.25)	Wood shavings	1	1.3×10^2	0.06
	B SLH25(ColE1::Tn*5*)		2	1.7×10^1	—
			3	1.0×10^1	—
			5	ND	
			6		ND
			8	1.0×10^1	—
			10	ND	

[a] Numbers in parentheses represent the initial A/B ratio at the time of aerosol release.
[b] For wood shavings, 50-g samples were used. For water, refuse water obtained from a chicken watering trough was used; when clean water was tested, all cultures were negative within 3 h (experiments 6, 7, and 8).
[c] —, There were fewer than 10 colonies to evaluate.
[d] ND, Not detectable (<10/g).
[e] NQ, Positive but not quantifiable.
[f] Cultures on days 13, 16, and 20 were negative.

the longest times. The laboratory strain persisted for a maximum of 10 days, and the wild-type strain could be recovered at a low titer ($<10^3$/g) through 20 days (Table 2).

Despite concentration, no organisms could be recovered from fresh water placed in the experimental room of the barn even on the day of bacterial release. On one occasion, bacteria were recovered from refuse water which had been removed from the chicken watering trough and placed in the experimental room (Table 2). Low titers of χ1666 (up to 5×10^1/ml) were obtained through 8 days, and low titers of SLH25 (up to 5.5×10^2/ml) were obtained through 10 days. Low numbers of test organisms were also recovered sporadically for a maximum of 8 days from flies trapped on gummed fly paper in two of the four experiments performed in the barn (experiments 5 and 8).

Relative recoveries of wild-type versus laboratory *E. coli* strains with and without ColE1::Tn*5*. In many of the initial samples, the total bacterial counts could be estimated, but no distinction between the two strains could be made. When numbers permitted, the relative amounts of each member of the pair were determined for comparison of survival. In both environments, the naturally occurring strain appeared to have a survival advantage. Whereas the ratios of χ1666 to SLH25 CFU released ranged from 0.1 to 0.53, the ratios of subsequent samples showed a marked decline (Fig. 1 and 2, experiments 4, 5, and 8; Table 3). We did note, however, that the χ1666/SLH25 recovery ratio in the office was markedly higher on paper (0.35) than on other substances (range, 0.04 to 0.13; data not shown). There was a 5- to 10-fold higher recovery of the wild-type strain from the skin and clothing of research personnel. A similar relationship was also observed for recoveries from water and wood chips (Table 2, experiments 5 and 8) and from flies.

In the office experiments, assessment of the effect of the ColE1 plasmid on the survival of the host strain was hindered by the low numbers recovered. In the barn, where more CFU were recovered by the different methods, differences between the test strains suggested that the plasmid provided some survival advantage, particularly in the wild-type host. This conclusion was supported by a decline in the ratios of plasmidless to plasmid-containing strains in the airborne state during the first 3 h (Fig. 1, experiments 6 and 7) as well as on GSC (Fig. 2, experiment 7). Certain surface types, particularly synthetic "cloth" (grain sacks) and grime-covered metal, supported more plasmid-bearing derivatives (Table 3). This was also true for wood chips (Table 2, experiment 7).

TABLE 3. Survival of strain pairs recovered from surfaces in the barn[a]

Surface (orientation)[b]	χ1666 (A) and SLH25 (B) (0.53)[c]		χ1666 (A) and χ1666(ColE1::Tn5) (B) (1.1)[c]		SLH25 (A) and SLH25(ColE1::Tn5) (B) (0.46)[c]		χ1666(ColE1::Tn5) (A) and SLH25(ColE1::Tn5) (B) (0.25)[c]	
	A/B ratio	h to LPC[d] (A/B)	A/B ratio	h to LPC (A/B)	A/B ratio[e]	h to LPC (A/B)	A/B ratio[f]	h to LPC (A/B)
Wood (V)	0.18	48/24	0.72	3/0.1	0.29	1/1	0.28	1/3
Wood (H)	0.14	48/48	0.72	4/4	0.39	3/3	0.04	0.5/4
Grain sack (H)	<0.10	72/72	0.28	2/3	0.27	4/4	0.03	1/4
Insulation (H)	0.12	24/48	0.90	3/3	0.42	0.5/0.5	0.02	0.17/≥3 < 5
Paper towelling (H)	0.04	1/5	0.69	4/4	0.41	2/4	0.03	1/3
Window glass (V)	0.11	5/5	—[g]	0.1/0.5	0.73	0.2/0.2	0.05	0.3/0.5
Metal[h] (H)	NQ[i]	120/240	0.49	2/240	0.19	3/4	0.06	4/4
Metal (V)	0.12	3/24	—	0.5/0.5	0.29	0.5/0.5	0.06	1/4
Wallboard (painted) (V)	0.16	3/48[j]	1.75	4/4	0.31	0.5/4	0.07	2/4
Ceiling (painted wallboard) (H)	0.14	6/24	0.73	3/0.5	0.29	4/0.3	0.01	2/2
Floor[k] (H)	NQ	72/240	NQ	<24/72	NQ	4/96	0.07	24/72

[a] Calculated from all assayable contact cultures taken over the entire test period.
[b] V, Vertical; H, horizontal.
[c] Numbers in parentheses represent the initial A/B ratio at the time of aerosol release.
[d] LPC, Last positive culture. h, Hours.
[e] Plates placed within a radius of 10 ft (ca. 3.05 m) outside the test room had an A/B ratio of 0.26.
[f] Plates placed within a radius of 10 ft (ca. 3.05 m) outside the test room had an A/B ratio of 0.03.
[g] —, There were fewer than 20 colonies to evaluate.
[h] Covered with grime.
[i] NQ, Not quantifiable.
[j] Two CFU were recovered on day 10.
[k] Wet and dry wood and cement surfaces.

DISCUSSION

While these experiments are limited, they do provide a general indication of what might be expected from the release of bacteria in an environment where ambient temperature and relative humidity conditions prevail and where different surfaces are available for settling of the organisms. We maximized the retention of bacterial particles within both environments by keeping windows and doors shut and air conditioning and ventilation systems closed. Since these systems function to remove and filter air, we would therefore expect the actual recovery and perhaps the survival of bacteria to be much lower if these systems were operational.

We observed the same rapid aerosol die-offs that have been reported previously with enclosed chambers (13; Hunt, Ph.D. thesis). The somewhat longer survivals found in the barn were perhaps related to the higher humidities (61 to 82%) (Hunt, Ph.D. thesis). Once they had settled out, however, organisms remained viable for hours or days, depending on the surface type. In the laboratory office, viability was generally limited to a few hours on all surfaces tested (Table 4). However, both the laboratory *E. coli* K-12 and wild-type *E. coli* strains remained viable on paper through 21 h. In the barn, depending on the experiment and surfaces tested, bacteria could be detected from 3 to 20 days after their release. Notably higher recoveries were obtained from grime-covered surfaces, especially those retaining moisture, such as wet cement and damp floors. Unvarnished wood and wood products appeared to have a particular affinity for supporting the viability of laboratory *E. coli* as well as the fecal strain. Wood shavings (used as animal bedding) sustained bacteria for relatively long periods of time (10 to 20 days). Survival and persistence in an environment, then, appear to be dependent upon the amounts and types of materials present there.

TABLE 4. Maximum time of detection (all experiments)[a]

Strain	Maximum time of detection (source) in:	
	Office	Barn
χ1666	4 h (Plastic)	10 days (Wood shavings)
χ1666(ColE1::Tn5)	21 h (Paper)	3 days (Dirt-covered concrete)
SLH25	30 min (Carpet)	20 days (Wood shavings)
SLH25(ColE1::Tn5)	21 h (Paper)	7 days (Wood shavings)

[a] For comparison of paired strains in the same experiment, see Fig. 1 and 2.

The poorer recovery of the laboratory strain as compared with that of the natural strain is consistent with the findings of Webb and Walker, who noted that auxotrophic mutants were more susceptible to dehydration than were their prototrophic parents (15). Like Hunt (Ph.D. thesis), who compared the survival of laboratory and naturally occurring *E. coli* strains in a static aerosol chamber, we found a prolonged survival of the wild-type strain and universal survival at higher humidity levels. However, in both the office and barn environments, we were able to detect low levels of the laboratory strain χ1666 (with or without a plasmid), in the aerosol state for much longer periods (up to 4 h) (Fig. 2) than previously reported.

In the four experiments which tested the effect of Col E1::Tn*5*, the plasmid provided only a slight or a short-term survival enhancement in laboratory strain χ1666. Stronger evidence for an improved survival of the plasmid-bearing derivative was seen with the wild-type strain (SLH25) in the barn environment (Table 3, experiment 7). A similar effect was also observed for recoveries from wood shavings (Table 2, experiment 7). These findings could presumably be attributed to the presence of either the plasmid or the transposon. A growth advantage of cells having Tn*5* has been shown in chemostats (2). However, Hunt (Ph.D. thesis) observed a survival advantage of laboratory *E. coli* HB101 bearing a ColE1 derivative, pBR-β-T, which bears no such transpo-

son. This advantage was confined to early samplings (i.e., up to 30 min) and appeared to be dependent on a higher humidity level. Likewise, Koniukhov et al. demonstrated an increased survival capacity of *E. coli* K-12 bearing plasmid pSA50 after aerosol dispersal and correlated this with the appearance of two new molecular weight fractions in the outer membrane (7).

These studies of aerosol release in a laboratory office and a barn indicate that *E. coli* can persist in these environments for appreciable periods of time. However, in no case was any test bacterium detected after 20 days, indicating an overall failure of either strain to thrive in these environments.

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