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A Stable Bioluminescent Construct of *Escherichia coli* O157:H7 for Hazard Assessments of Long-Term Survival in the Environment

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A chromosomally *lux*-marked (Tn*5 luxCDABE*) strain of nontoxigenic *Escherichia coli* O157:H7 was constructed by transposon mutagenesis and shown to have retained the O157, H7, and intimin phenotypes. The survival characteristics of this strain in the experiments performed (soil at −5, −100, and −1,500 kPa matric potential and artificial groundwater) were indistinguishable from the wild-type strain. Evaluation of potential luminescence was found to be a rapid, cheap, and quantitative measure of viable *E. coli* O157:H7 Tn*5 luxCDABE* populations in environmental samples. In the survival studies, bioluminescence of the starved populations of *E. coli* O157:H7 Tn*5 luxCDABE* could be reactivated to the original levels of light emission, suggesting that these populations remain viable and potentially infective to humans. The attributes of the construct offer a cheap and low-risk substitute to the use of verocytotoxin-producing *E. coli* O157:H7 in long-term survival studies.

The incidence of food poisoning outbreaks has increased in recent years, with *Escherichia coli* O157:H7 emerging as a pathogen of increasing public health concern. The infective dose of *E. coli* O157:H7 in humans has been estimated to be very low (11), and infection can result in a wide range of clinical manifestations, including life-threatening hemolytic-uremic syndrome (37). *E. coli* O157:H7 resides harmlessly in the digestive system of cattle and many other animals and is excreted in animal feces, from where it can enter the environment (15, 45). Recent reports have attributed *E. coli* O157:H7 outbreaks to a diverse range of sources, including swimming pools, water supplies, raw vegetables, and, more commonly, beef products (15, 28).

There is little information regarding the behavior and metabolic status of *E. coli* O157:H7 in the environment, although some reports suggest the potential for considerable survival in cattle feces, soil, and water (9, 14, 18, 42). Vertical transmission of *E. coli* O157:H7 from cattle feces through soil has also been demonstrated (10), and this finding highlights a possible transmission route leading to the contamination of private drinking water. All of these studies, however, have utilized culture-based methods, which rely on disruption of cells from environmental material followed by plating on selective media, such as cefixime- and potassium tellurite-containing sorbitol MacConkey agar (6). These approaches will, however, fail to target *E. coli* O157:H7 populations that may be in a viable but nonculturable state. The occurrence of the viable but nonculturable state in enteric bacteria is highly disputed by some (3), while other reports suggest it does occur in *E. coli* commensal and O157:H7 populations in water and under saline conditions (21, 31, 42). Additionally, the ability to disrupt bacterial cells which are tightly adhered to soil particles may also be inefficient, leading to an underestimation of target population size. Molecular techniques, such as quantitative PCR, eliminate some of the biases of culture-based methods for estimation of pathogen abundance in soil, although, in most cases, an enrichment step is still required (22). Despite this, PCR-based approaches rely on direct extraction of nucleic acids or cells (followed by nucleic acid extraction) from soil, which may also be biased depending on the extraction method employed (23). The possibility of PCR detection of intact DNA from nonviable pathogens also limits the value of this approach (16).

As an alternative approach, the *lux* genes, which encode bioluminescence, have been successfully used in studies of the fate of microorganisms in the environment, including soil (33). Use of constructs which have been chromosomally marked with the full *luxCDABE* cassette offer the same advantages for localization of the target organism as marking with jellyfish green fluorescent protein (GFP) (33) but also offer potential assessment of metabolic activity. Although a GFP-marked *E. coli* O157:H7 strain has been described recently (14), the fluorescent phenotype of that strain was not used other than for verification of culturable plate counts. In contrast to the GFP fluorescence phenotype, which does not change on starvation or entry into the viable but nonculturable state, the bioluminescence phenotype is dependent on the energy status of the cell (39). Application of *lux* marker systems has therefore enabled measurement of population activity (26), detection of viable but nonculturable cells (7), and nonextractive estimation of active biomass of target populations in soil (25, 27).

The present study first describes the construction of a chromosomally *lux*-marked nontoxigenic strain of *E. coli* O157:H7. Second, the applicability of this stable chromosomally *lux*-

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TABLE 1. Composition of groundwater[a]

Constituent	Concn (mM)
NaCl	0.07
$MgCl_2$	0.07
$MgSO_4$	0.07
$CaSO_4$	0.045
K_2SO_4	0.02
$(NH_4)_2NO_3$	0.02
KH_2PO_4	0.01

[a] The pH was 7.0.

marked strain for survival studies in soil and artificial groundwater samples is demonstrated. A potential luminescence assay has been applied to provide a quick in situ estimation of the size of potentially active bioluminescent *E. coli* O157:H7 populations.

MATERIALS AND METHODS

Bacterial strain and culture conditions. *E. coli* O157:H7 strain 3704 was kindly provided by Fiona Thompson-Carter (*E. coli* reference laboratory, University of Aberdeen, United Kingdom). The strain was originally isolated from a farm drain and has been proven to be nontoxigenic due to the absence of toxin activity (by Verocell assay) and toxin genes (by PCR) (F. Thomson-Carter, unpublished observation). The strain and subsequent constructs were maintained on LB agar. Stock cultures were kept in 10% (vol/vol) glycerol at −80°C. Cell numbers were quantified on both sorbitol MacConkey agar (SMAC) (Oxoid Ltd., United Kingdom), and tryptone soy agar (TSA) (Oxoid Ltd., United Kingdom), at 37°C for 24 h to estimate differences due to sublethal injury (40).

Chromosomal *lux* marking of *E. coli* O157:H7. *E. coli* O157:H7 strain 3704 Tn*5* *luxCDABE* was constructed by biparental mating of a spontaneous rifampin-resistant mutant of *E. coli* O157:H7 strain 3704 with a donor strain, followed by suicide plasmid delivery and transposon mutagenesis. The donor strain, *E. coli* S17 λ*pir luxCDABE* Km2, was a kind gift from P. Hill (University of Nottingham, United Kingdom) and contains the *luxCDABE* cassette from *Photorhabdus luminescens* and the antibiotic resistance genes for ampicillin and kanamycin (43). The rifampin-resistant mutant of *E. coli* O157:H7 strain 3704 was made by plating serial dilutions of an overnight culture onto Luria-Bertani (LB) plates containing rifampin (100 μg ml^{-1}). Transconjugants in which Tn*5* *luxCDABE* had inserted into the chromosome were initially selected on the basis of growth on LB containing rifampin (100 μg ml^{-1}) and kanamycin (50 μg ml^{-1}) and then by visible bioluminescence in the dark. The absence of the plasmid (which conferred ampicillin resistance) was confirmed by small-scale plasmid DNA preparations and by the lack of growth of the transconjugants on LB plates containing ampicillin (50 μg ml^{-1}).

The stability of the *lux* phenotype was examined by successive subculturing of the selected *E. coli* O157:H7 strain 3704 Tn*5* *luxCDABE* in LB broth without addition of kanamycin and subsequent confirmation of colony growth on LB agar with versus without kanamycin (50 μg ml^{-1}). To confirm that mutagenesis had not disrupted the O157, H7, or intimin phenotype of the strain, multiplex PCR as described by Campbell et al. (4) was performed.

Southern blot conditions. Genomic DNA from the donor, host, and chromosomally *lux*-marked transconjugants was isolated according to standard procedures (34) and digested to completion with *Nde*I. The digested genomic DNA was probed with a 1,273-bp fragment of the *luxCDABE* cassette (containing a single *Nde*I site) which had been amplified with the primer pair JR42 (5′-CGC TGT CGG AAA TTA TAC GG-3′) and JR43 (5′-GTT ACG GTA AAT GTC GTA GG-3′). The specific PCR conditions used to generate the *lux* fragment were 95°C for 1 min 30 s, then 29 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. The probe was purified from a 1% agarose gel and labeled according to the manufacturer's instructions (ECL direct nucleic acid labeling and detection system; Amersham Pharmacia, United Kingdom). Southern blotting conditions were as recommended by the manufacturer of the labeling kit.

Growth experiments comparing chromosomally *lux*-marked and wild-type *E. coli* O157:H7. Batch culture growth experiments were carried out in triplicate in tryptone soy broth (TSB) (Oxoid Ltd., United Kingdom). The cultures were incubated at 37°C at 200 rpm, and 1-ml samples were removed at regular intervals for analysis of optical density at 600 nm and bioluminescence (relative light units [RLU]). Bioluminescence was measured with a Jade luminometer (Labtech International Ltd., United Kingdom).

Potential bioluminescence assay. A slight modification of the method by Duncan et al. (7) was used. Briefly, 9 ml of warm TSB (37°C) was inoculated with either pure culture or inoculated groundwater (1 ml) or inoculated soil (1 g) from the survival studies described below. The mixture was incubated at 37°C with continuous shaking at 200 rpm for 30 min. After this activation step, luminescence was measured from 1-ml undiluted aliquots of the mixture as described above. When potential luminescence was measured from soil, aliquots of the TSB-sample mixture (1.5 ml) were first centrifuged at 4,000 × *g* for 5 s, and 1 ml of supernatant was used for luminescence measurements. Cell numbers were quantified with the remainder of the TSB-sample mixture.

Potential luminescence as an indicator of potentially active population size of *E. coli* O157:H7 in soil. To investigate potential luminescence as an indicator of the size of potentially active *E. coli* O157:H7 populations, stationary-phase (15-h) cultures (washed and resuspended in one-quarter-strength Ringer's solution) of chromosomally *lux*-marked *E. coli* O157:H7 were subjected to 10-fold dilutions in one-quarter-strength Ringer's solution. Aliquots (1 ml) of this dilution series were used for the potential luminescence assay described above. To investigate the response in soil, 0.8 ml of the dilution series was added to 10 g (fresh weight) of Insch soil ($n = 3$) to achieve a soil matric potential of −100 kPa, and the

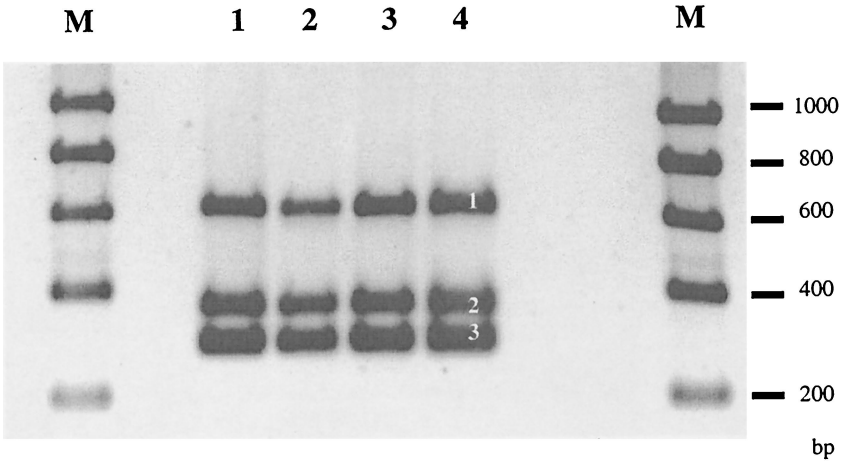

FIG. 1. Mutagenesis of wild-type strain (lanes 1 and 2) with the chromosomal insertion of a *lux* cassette did not interrupt the products for the H7 (band 1), intimin (band 2), or O157 (band 3) markers in the *lux*-marked mutant (lanes 3 and 4). Lanes M, Bioline Hyperladder I molecular size markers.

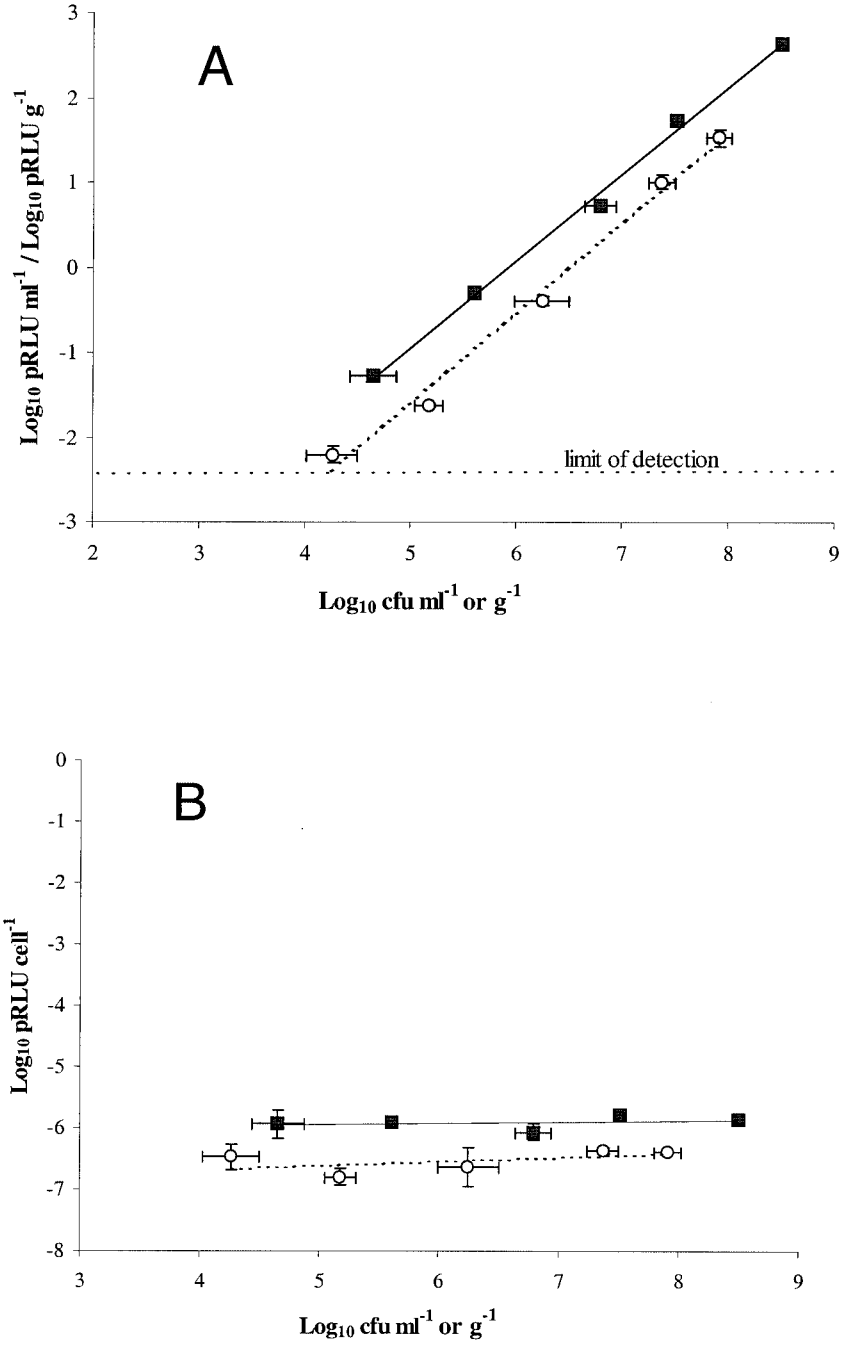

FIG. 2. (A) Potential luminescence response of a dilution series of a pure culture of chromosomally *lux*-marked *E. coli* O157:H7 in one-quarter-strength Ringer's solution (■) and when inoculated into Insch soil (○); (B) their corresponding potential luminescence values per cell. Data represent the mean of three replicates ± standard deviation.

potential luminescence assay was performed with the inoculated soil. The response of the potential luminescence to starved populations of *E. coli* O157:H7 was also tested by incubating the washed stationary-phase culture for up to 15 days at 15°C. The potential luminescence assay was performed with and without the inclusion of nalidixic acid (10 μg ml^{-1}) in order to investigate whether growth could take place during the course of the assay. The remainder of the dilution series was used for estimation of cell numbers on SMAC and TSA.

Validation of substitute strain for survival studies in natural water samples. The survival responses of the wild-type and the *lux*-marked construct were also compared in sterile artificial groundwater. Both strains were grown to late exponential phase in TSB, washed twice with 1 volume of sterile artificial groundwater (Table 1), and incubated at 15°C in the dark for 70 days. Viable cell numbers were established at set time points by plate counts on SMAC and TSA following dilution in one-quarter-strength Ringer's solution. Potential luminescence of the chromosomally *lux*-marked strain was measured as described above.

Validation of marked strain as a substitute in a soil survival study. Topsoil (a sandy loam) was collected from Insch, northeastern Scotland, sieved to 3 mm, oven dried (105°C), and stored at 4°C until required. A stationary-phase (15-h) culture of chromosomally *lux*-marked *E. coli* O157:H7 strain 3704 or wild-type strain 3704 was centrifuged for 10 min at 11,000 × *g*, after which cell pellets were

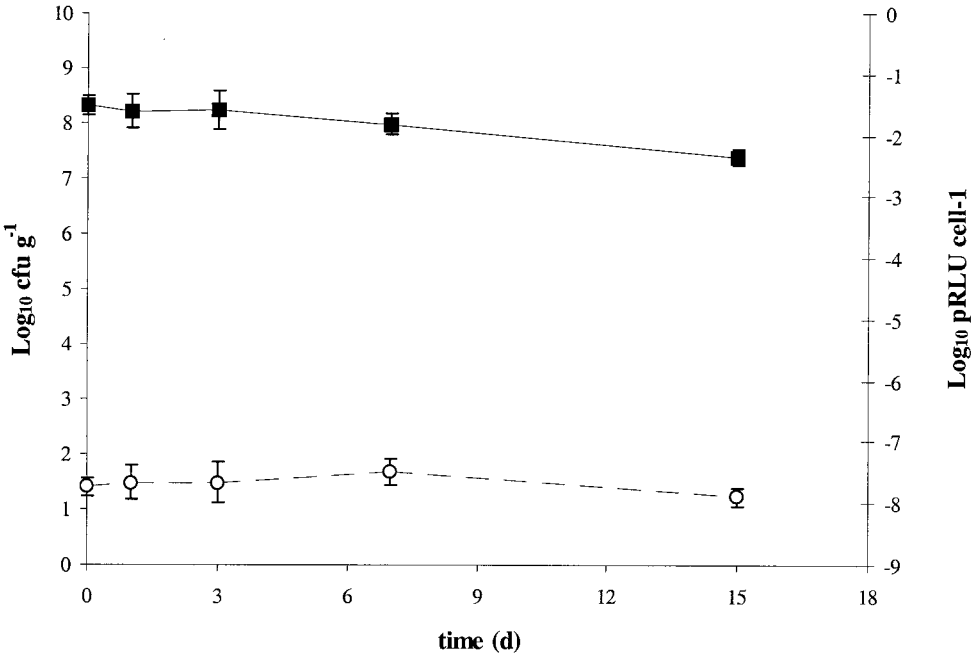

FIG. 3. Survival of chromosomally *lux*-marked strain to starvation in one-quarter-strength Ringer's solution for 15 days, as assessed by culturable cell counts (■) and potential luminescence (pRLU) per cell (○). Data represent the mean of three replicates ± standard deviation.

resuspended in one-quarter-strength Ringer's solution and left to starve for 24 h. A volume of resuspended cells was then added to sieved and dried soil to achieve soil matric potentials, after equilibration, of −5, −100, and −1,500 kPa. Matric potential was assessed by a combination of pressure plate and tension table measurements (30). The final matric potentials were prepared in accordance to the moisture release characteristics of the soil. The inoculated soil (30 g) was weighed into glass jars, which were sealed with parafilm and incubated at 15°C. Uninoculated control soil, which was equilibrated to the same matric potentials, was also included. The microcosms were sampled in triplicate after 1, 5, 10, 20, 35, 60, 90, and 158 days of incubation at 15°C. At each sampling point, potential luminescence of samples was measured with the methods described above. Numbers of chromosomally *lux*-marked *E. coli* O157:H7 were determined by serial dilution of soil in one-quarter-strength Ringer's solution and plating appropriate dilutions on SMAC and TSA.

Statistical analysis. One-way analysis of variance (with Excel 97) was performed to compare differences in specific growth rates between the wild-type and chromosomally *lux*-marked *E. coli* O157:H7. One-way analysis of variance was also performed to test differences in survival rates in the soil and water survival experiments at each time point. Differences were considered significant at the $P \leq 0.05$ level.

RESULTS

Characterization of chromosomally *lux*-marked against wild-type *E. coli* O157:H7. Batch culture experiments comparing the wild-type and the chromosomally *lux*-marked *E. coli* O157:H7 strain 3704 showed that *lux* marking slightly affected the specific growth rate at 37°C. The wild-type strain displayed a specific growth rate of 1.13 ± 0.03 h^{-1}, whereas the *lux*-marked strain grew slightly faster at 1.24 ± 0.01 h^{-1}. The difference was statistically significant ($P < 0.05$). Stability experiments involving successive subculturing of the conjugant in the absence of antibiotics found that the *lux* phenotype was stable for over 100 generations (data not shown). The insertion of the *lux* cassette had not disrupted the genes for production of the O157, H7, or intimin phenotype, as shown by the identical sizes of the PCR products for these markers (Fig. 1). Southern analysis confirmed a single chromosomal insertion of the *luxCDABE* cassette in *E. coli* O157:H7 strain 3704. Two bands were detected in the transconjugants due to the presence of the *Nde*I site in the probe fragment. However, only one band was present in the donor strain as the plasmid containing the *luxCDABE* cassette includes only one restriction site for *Nde*I. No bands were detected from the wild-type *E. coli* O157:H7 strain 3704 (data not shown).

Potential luminescence as an indicator of potentially active population size of *E. coli* O157:H7. Potential luminescence was proportional to the number of chromosomally *lux*-marked *E. coli* O157:H7, either as pure culture washed in one-quarter-strength Ringer's solution or as a soil inoculum (Fig. 2A). In these experiments, increasing numbers of cells resulted in a proportional increase in luminescence within the 30-min assay period, i.e., potential luminescence per cell was the same over a range of cell densities between 10^4 and 10^8 CFU per ml or per g of sample (Fig. 2B). The results suggested that the potential luminescence assay was sensitive enough to detect $>4.0 \times 10^3$ CFU ml^{-1} when cells were presented in a clear matrix, in this case, one-quarter-strength Ringer's solution. Similarly, although the presence of soil had a significant masking effect, the assay could detect $>1.5 \times 10^4$ CFU per g of Insch soil. Starvation of the cells over 15 days did not result in a significant loss of potential luminescence per cell (Fig. 3). There was no increase in cell numbers during the course of potential luminescence assays ($P > 0.05$). In addition, inclusion of nalidixic acid (10 μg ml^{-1}) to inhibit cell division had no statistically significant effect on potential luminescence (data not shown).

Survival of wild-type and *lux*-marked *E. coli* O157:H7 in artificial groundwater. The population sizes of both the wild-

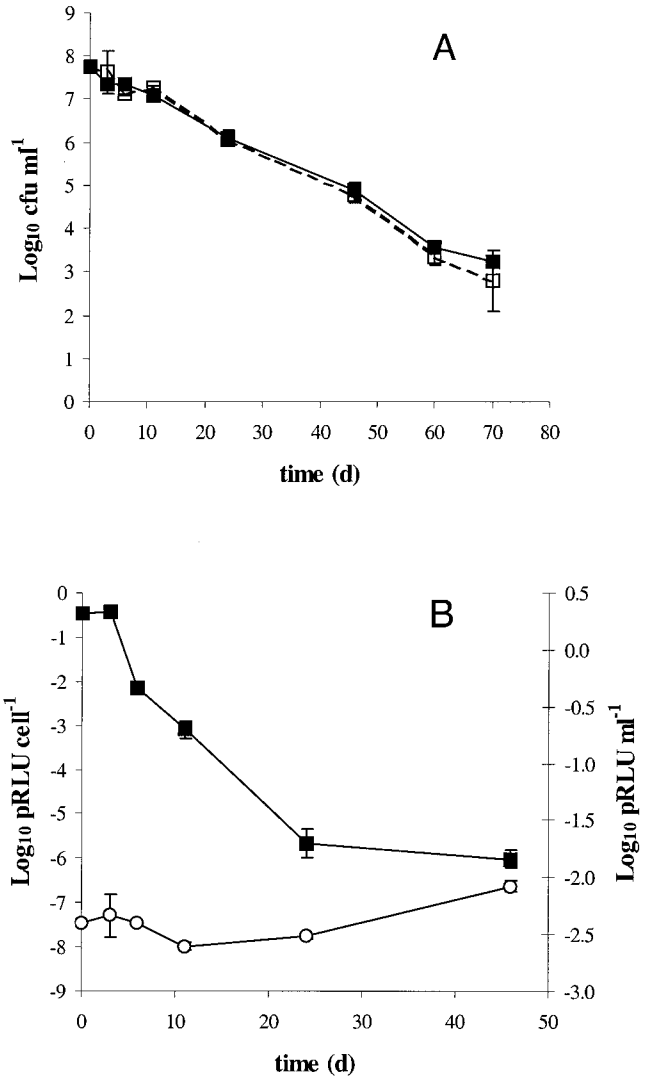

FIG. 4. (A) Survival of wild-type (■) and chromosomally *lux*-marked (□) *E. coli* O157:H7 in artificial groundwater at 15°C. (B) The potential luminescence (pRLU) of the *lux*-marked strain (■) dropped at an equivalent rate to the cell numbers, as seen from the potential luminescence per cell (○) response. Only data until day 45 are shown, as potential luminescence values dropped below the detection level after this time point. Data represent the mean of three replicates ± standard deviation.

type and the chromosomally *lux*-marked strain declined by approximately 5 log units over the course of the 70 days of incubation in artificial groundwater at 15°C (Fig. 4A). There was no statistical difference between the population sizes of the strains at any of the time points ($P > 0.05$) or of the cell numbers retrieved on either TSA and SMAC.

Survival of *E. coli* O157:H7 and the *lux*-marked strain in soil at different matric potentials. The effects of soil matric potential on survival of wild-type and chromosomally *lux*-marked *E. coli* O157:H7 strain 3704 was investigated in Insch soil at 15°C equilibrated to matric potentials of −5 kPa, −100 kPa, and −1,500 kPa (Fig. 5). Both wild-type and *lux*-marked *E. coli* O157:H7 populations declined relatively quickly (by approximately 3.5 log units) during the first 35 days of the experiment. Population decline was less rapid for the remainder of the 90-day experiment. There was no statistically conclusive evidence with regards to different soil matric potentials influencing the survival characteristics of *E. coli* O157:H7 populations (Table 2). Additionally, although suggestive at some sampling points, there was no conclusive statistical evidence for a difference between the wild-type and *lux*-marked strains.

Potential luminescence as an indicator of metabolic activity in survival studies. In both the artificial groundwater experiment (Fig. 4B) and at all matric potentials used in Insch soil (Fig. 6), values of potential luminescence per cell remained relatively constant. This indicated that there was no decrease of potential metabolic activity in the cells over time. Potential luminescence values in the assays dropped below the detection limit after 45 days in artificial groundwater and after 35 days in the soil matric potential experiments due to the decreases in total cell number, and potential luminescence per cell could thus not be calculated for these time points. In addition, the slight increases in potential luminescence at the last time points shown in each of these figures are most likely an artifact of the combination of very low potential luminescence values and small cell numbers in these samples.

DISCUSSION

In the European Community, verocytotoxin-producing *E. coli* O157:H7 has recently been reclassified from Hazard Group 2 to a Hazard Group 3 pathogen in response to a number of laboratory-acquired infections (5, 8). This level of classification limits the ability of most European research laboratories to conduct studies with the toxigenic organism. Nontoxigenic isolates of *E. coli* O157:H7 provide an alternative approach to study pathogen behavior and movement. Such nontoxigenic strains appear to possess the same characteristics as toxigenic isolates with the exception of the genes coding for the cytotoxins *stx1* and *stx2* (35). Whether the presence of the cytotoxins constitutes an advantage in survival and transport of *E. coli* O157:H7 in the environment is debatable. The toxicity of Shiga toxin and similar toxins to eukaryotic cells has been well documented, and it has been speculated that this cytotoxicity may aid the survival of *E. coli* O157 in eukaryotic cells (17, 19). In a recent, comprehensive study of the survival of various O157:H7 isolates in manures and manure slurries, however, identical or very similar survival patterns were obtained for toxigenic and nontoxigenic strains (18). The use of nontoxigenic isolates can thus be viewed as a valid alternative approach to study pathogen behavior and movement in environmental samples.

Use of a *lux*-marked *E. coli* O157:H7 to monitor adherence to food has been reported previously (36). The use of this construct enabled real-time visualization of plasmid-marked *E. coli* O157:H7 adherence to animal carcass tissue and clearly highlighted the potential values of a *lux* marker system in *E. coli* O157:H7 survival studies. Due to potentially high copy numbers, plasmid *lux*-marked constructs may exhibit higher luminescence than chromosomally *lux*-marked constructs (1). The disadvantage of such constructs, however, is that a selective pressure (such as addition of antibiotics) must be applied to maintain the plasmid. Such constructs are, therefore, only suitable for short-term studies where plasmid loss is unlikely to

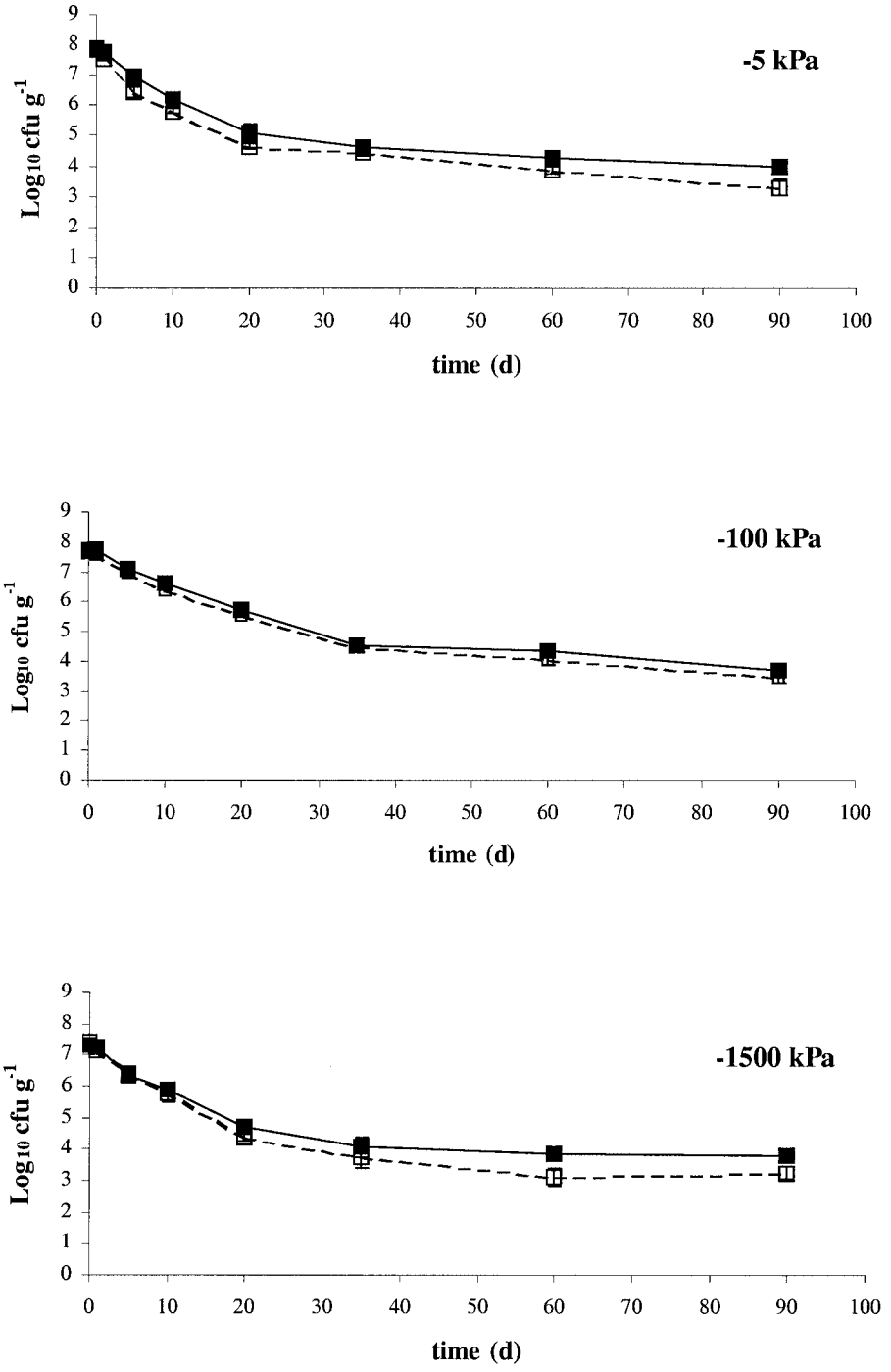

FIG. 5. Survival of wild-type (■) and chromosomally *lux*-marked (□) *E. coli* O157:H7 in Insch soil at 15°C and matric potentials of −5 kPa, −100 kPa, and −1,500 kPa. Data represent the mean of three replicates ± standard deviation.

occur. In contrast, use of stable chromosomally *lux*-marked constructs, such as that reported in this study, eliminates the requirement of applying a selective pressure and is thus desirable for long-term survival experiments. Although the construction of a chromosomally *lux*-marked *E. coli* O157:H7 strain has been reported previously (41), this was a *luxAB* mutant, which is dependent on addition of *n*-decanol for display of the luminescent phenotype. The construct described in this study alleviates this need for additions prior to analysis.

Although there was a slight difference in growth rate between the chromosomally *lux*-marked and the wild-type *E. coli* O157:H7 strain at 37°C, this difference was not noted in the long-term survival experiments. In experiments with soil and artificial groundwater, there was no difference in survival be-

TABLE 2. Recovery of *E. coli* strains from spiked Insch soil at 15°C and different matric potentials[a]

Day	Recovery (% of inoculum) at potential (kpa):					
	−5		−100		−1500	
	WT	*clux*	WT	*clux*	WT	*clux*
0	100	100	100	100	100	100
1	74.7 ab	46.7 a	103.3 b	77.6 ab	86.3 b	49.9 a
5	12.1 ab	3.9 a	24.2 c	20.3 bc	11.6 a	9.4 a
10	1.94 ab	0.91 a	8.74 c	5.00 d	4.06 bd	2.61 ab
20	0.190 ab	0.061 a	0.958 c	0.724 d	0.263 b	0.097 a
35	0.059 a	0.041 a	0.067 a	0.061 a	0.064 a	0.024 b
60	0.0271 ab	0.0105 c	0.0437 d	0.0220 a	0.0335 bd	0.0058 c
90	0.0121 a	0.0031 a	0.0094 a	0.0063 a	0.0357 b	0.0071 a

[a] Values with different letters within a row were significantly different. WT, wild type; *clux*, chromosomally *lux* marked.

havior between the wild-type and chromosomally *lux*-marked strain. These findings support the validity of the construct as a substitute for the wild-type strain for long-term experiments. In addition, the observed survival rates correspond well with published data of survival of toxigenic O157:H7 strains in similar environmental samples (14, 24, 29, 42). Although the experiments presented here have been conducted in essentially sterile environments in order to provide preliminary validation for the use of this *lux*-marked construct, recent findings also suggested the validity of application in nonsterile environmental samples (2).

The data obtained here suggest that contrasting matric potentials did not influence the survival of *E. coli* O157:H7 in Insch soil. Although matric potential is thought to affect bacterial movement below −20 kPa and, similarly, decrease bacterial activity below −50 kPa (44), no difference in survival of *E. coli* O157:H7 at matric potentials between field capacity (−5 kPa) and wilting point (−1,500 kPa) was noted. Similar observations were made by Meikle et al. (25) and Turnbull et al. (38) when survival of *Pseudomonas fluorescens* in soil was studied. In the study by Meikle et al. (25), some indication that increased matric stress reduces bacterial survival was found, but, as in this study, these indications were not confirmed by statistical differences. Although survival of *E. coli* O157:H7 may be similar in soil at most realistic matric potentials, the differences in matric potential will most certainly have an effect on the localization of bacteria within the soil in the event of a sudden rainfall. In the drier soils, the cells will be translocated into the smaller pores within the soil matrix (32), which will limit their potential transport by leaching. Thus, although there may be an equal risk of infection by direct ingestion of soils of different matric potentials, the infection risk through leaching should be investigated further.

Potential luminescence of *lux*-marked *E. coli* O157:H7 introduced into artificial groundwater and soil was used to assess the viability and metabolic activity of the strain in situ. Although the cell densities needed for adequate detection of potential luminescence may appear high for environmental samples, such cell densities have been shown to occur in fecal material of actively shedding cattle (45) and may thus be within a similar range in top layers of soil and/or soil leachates. In both the artificial groundwater and the soil survival experiments, final values of potential luminescence per cell were similar, irrespective of the incubation time of the chromosomally *lux*-marked *E. coli* O157:H7 populations in soil. This suggested that *E. coli* O157:H7 populations were capable of reactivation to similar metabolic levels as when they had been introduced into soil or water.

Despite the possibility of experiencing starvation conditions, our results suggest there was no noticeable effect of starvation or matric stress on the potential metabolic activity of the cells. These findings may bear important considerations with regard to ingestion of environmental material contaminated with *E. coli* O157:H7. The capacity for an ingested starved *E. coli* O157:H7 population to reactivate may have implications regarding expression of virulence factors (e.g., intimin binding gene, Shiga toxin genes) which may influence the likelihood or severity of infection.

Currently, there are no data that document the effects of starvation in soil on the virulence traits of *E. coli* O157:H7. Starvation of *E. coli* O157:H7 in water has, however, been shown to influence the expression of the O157 antigen (12) and the development of a chlorine-resistant phenotype (20). Other studies also point to the importance of the physiological condition of *E. coli* O157:H7 with regard to the expression of cellular adhesion factors (13). The potential luminescence assay described here does not rely on cultivation of cells and requires only an activation step and could therefore be used for the fast quantification of nonoptimal metabolism of starved cells to further investigate the likelihood of infection arising from *E. coli* O157:H7 in environmental material. In addition, although not observed within the experimental framework of this validation study, trends of potential luminescence over the course of survival studies could be also potentially be used to quantify metabolic states such as sublethal injury and the viable but nonculturable response.

In this study, we successfully engineered a chromosomally *lux*-marked *E. coli* O157:H7 construct. With this construct, an assay was developed which enabled predictions of the size of potentially active populations of chromosomally *lux*-marked *E. coli* O157:H7 to be made without the limitations of the established detection methods. The in situ, metabolically linked nature of the luminescence-based assay, eliminated the requirement of cell extraction and provided the ability to discriminate viable populations of chromosomally *lux*-marked *E. coli* O157:H7 from nonviable cells. This assay provides estimates of chromosomally *lux*-marked *E. coli* O157:H7 relative population sizes within 30 min, which is advantageous over alternative, time-consuming techniques such as quantitative PCR and culture-based techniques. The attributes of this con-

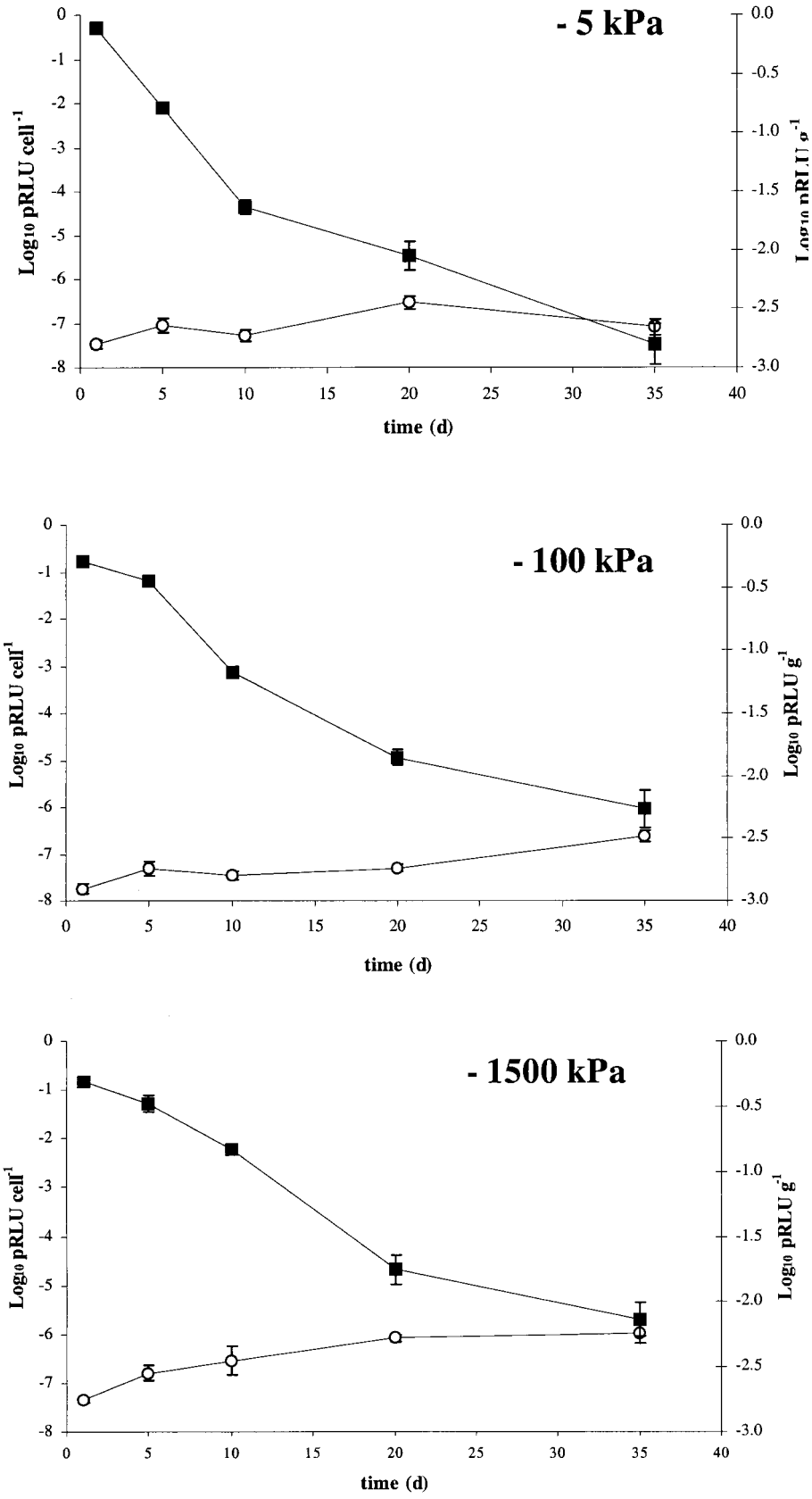

FIG. 6. Corresponding values (to the cell numbers in Fig. 5) of potential luminescence (■) and luminescence per cell (○) in microcosms of Insch soil at 15°C and matric potentials of −5, −100, and −1,500 kPa, spiked with a culture of chromosomally *lux*-marked *E. coli* O157:H7. Data shown are for the first 35 days, as potential luminescence dropped below the detection limit after this time point. Data represent the mean of three replicates ± standard deviation.

struct may thus provide a potentially useful tool for future studies of survival and transfer of *E. coli* O157:H7 in the environment.

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