Journal of Food Protection, Vol. 63, No. 9, 2000, Pages 1173–1178

Studies on the Growth of *Escherichia coli* O157:H7 Strains at 45.5°C

JASON FERENC, JASON OLIVER, RUTH WITKOWSKI, LYNNE McLANDSBOROUGH, AND ROBERT E. LEVIN*

Department of Food Science, Agricultural Experiment Station, University of Massachusetts, Amherst, Massachusetts 01003, USA

MS 99-255: Received 27 August 1999/Accepted 19 March 2000

ABSTRACT

The objectives of the present report were to examine the ability of 18 strains of *Escherichia coli* O157:H7 to grow in EC broth at 42.4, 43.5, 44.5, and 45.5°C, and to document the incidence of phenotypic variants present in low numbers that are capable of growth at 45.5°C in EC broth. Among the 18 strains of *E. coli* O157:H7 studied, only 3 were capable of producing turbid growth with gas formation in EC broth at 45.5°C with 1×10^2 initial CFU/ml. Higher initial densities of CFU resulted in turbid growth and gas formation in EC broth at 45.5°C with all strains. The presence of bile salts #3 in EC broth was found to be inhibitory at 45.5° C. All 18 strains were found to be capable of growth at 45.5°C in nonselective media. The ability of at least one sensitive strain to grow in EC broth at 45.5°C was found to be dependent on the initial number of CFU/ml. Prior growth of cells of a sensitive strain in EC broth at 45.5°C from a cell density of 2.0×10^7 to 8.0×10^7 CFU/ml followed by removal of cells and reinoculation at a cell density of 2.0×10^6 CFU/ml resulted in growth at 45.5°C that did not occur without such conditioning of the inhibitory medium. These results indicate that the ability of most strains of *E. coli* O157:H7 to grow in EC broth at 45.5°C is dependent on the initial density of CFU and that at low densities of CFU the ability to initiate growth is dependent on either low numbers of phenotypic variants tolerant to the presence of bile salts #3 in EC broth at 45.5°C or to conditioning of the medium with prior elevated numbers of cells.

Doyle and Schoeni *(1)* were the first to report that *Escherichia coli* O157:H7 was unable to grow at 45.5°C. These authors used trypticase soy broth (TSB) with 1×10^3 initial CFU per ml in shake flasks. Raghubeer and Matches *(7)* later reported that *E. coli* O157:H7 was unable to grow in EC broth at 44.5°C when culture tubes were inoculated with 10 CFU/ml. Both studies involved the examination of just one culture of *E. coli* O157:H7. Because growth with gas production within 48 h in EC broth at 44.5°C is presumptive for the presence of the fecal coliform *E. coli*, Raghubeer and Matches *(7)* concluded that O157:H7 strains would probably not be detected in normal screening for fecal coliforms with EC broth at 44.5°C. Palumbo et al. *(6)* found that all 23 *E. coli* O157:H7 strains examined grew at 45°C in brain heart infusion broth. They also reported that 3 of 23 of the O157:H7 strains failed to grow at 45°C in EC broth and that an additional 3 strains yielded variable results in EC broth at 45°C. They used an inoculum of about 10^3 CFU/ml. The present report examines the ability of 18 strains of *E. coli* O157:H7 to grow in EC broth at 42.4, 43.5, 44.5, and 45.5°C, and documents the incidence of phenotypic variants present in low numbers, capable of growth at 45.5°C in EC broth. This paper also presents data indicating that the ability of at least one isolate to initiate growth in EC broth at 45.5°C is dependent on the initial density of CFU.

MATERIALS AND METHODS

Source of cultures. *E. coli* strain GG was originally isolated from a soft-shell clam (*Mya arenaria*) by this laboratory and adheres to the major metabolic characteristics of a classic commensulate *E. coli* isolate (Indole +, methyl red +, Voges-Proskaur −, citrate −). In addition to producing a typical green metallic sheen on Levine's EMB agar and utilizing sorbitol, it produces turbidity and gas in 10 ml of EC broth at 45.5°C in 24 h with 1×10^2 initial CFU/ml. *Bacillus stearothermophilus* strain 65b was obtained from the culture collection of this Department and was used for the quantitative detection of bile salts #3 in EC broth after conditioning EC broth as described below. Additional cultures studied are listed in Table 1. Each *E. coli* O157:H7 strain studied produced a green metallic sheen on Levine's EMB agar, failed to utilize sorbitol on sorbitol–MacConkey agar, and was confirmed by slide agglutination to adhere to the O157:H7 serotype. Antisera were obtained from Difco Laboratories (Detroit, Mich.).

Routine culture methods. TSB was obtained from Becton Dickinson Microbiology Systems (Cockeysville, Md.). All other culture media were obtained from Difco. The initial determination of growth and gas production in EC broth at 42.4, 43.5, 44.5, and 45.5°C was performed by inoculating duplicate tubes containing 10 ml of EC broth and inverted gas vials with 10 μl (containing a total of 10^3 CFU) of appropriately diluted overnight EC broth cultures incubated at 32°C with rotary agitation (200 rpm). Incubation of EC broth tubes was static in covered waterbaths connected to thermistor control units (±0.1°C, Versa Therm proportional temperature controllers, model 2156; Cole-Parmer Instrument Co., Vernon Hills, Ill.). The number of CFU was estimated from the relationship of absorbance of cell suspensions at 600 nm (A_{600}) (using cuvettes having a light path of 1 cm) to CFU following the correlation of turbidity of cell suspensions (A_{600} readings) with colony counts. CFUs were determined with surface spread plates of tryptic soy agar containing 0.5% glucose (TSA). Plates were inoculated in duplicate using a series of decimal dilutions (0.1 ml inoculum) of an overnight culture of TSB containing 0.5% glucose (TSB).

* Author for correspondence. Tel: 413-545-0187; Fax: 413-545-1262; E-mail: relevin@foodsci.umass.edu.

TABLE 1. *Designations and origins of* E. coli *strains used*[a]

Strain designation (this study)	Original strain designation	Serotype	Source
GG	GG	—	Soft shell clam, this lab
C9490	C9490	O157:H7	CDC
C9490$^+$	C9490$^+$	O157:H7	Strain C9490, this lab
933	EDL 933	O157:H7	H. Schmidt
E4	ATCC 35150	O157:H7	Vicam L. P.
E5	MF6707A	O157:H7	Vicam L. P.
E6	MF1847	O157:H7	Vicam L. P.
E7	A9124-1	O157:H7	Vicam L. P.
E7$^+$	E7$^+$	O157:H7	Strain E7, this lab
E18	ATCC 43890	O157:H7	CDC
E18$^+$	E18$^+$	O157:H7	Strain E18, this lab
E20	ATCC 43894	O157:H7	CDC
E21	ATCC 43895	O157:H7	CDC
E59	PA#98E125	O157:H7	MDPH
E60	PA#97E249	O157:H7	MDPH
E61	PA#98E114	O157:H7	MDPH
E62	PA#98E85	O157:H7	MDPH
E63	PA#97E288	O157:H7	MDPH
E65	PA#98E66	O157:H7	MDPH
E66	PA#98E67	O157:H7	MDPH
E67	PA#98E64	O157:H7	MDPH
E68	PA#98E62	O157:H7	MDPH

[a] CDC, Centers for Disease Control and Prevention, Atlanta, GA 30333; ATCC, American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852-1776; MDPH, Massachusetts Department of Public Health, 305 South St., Jamaica Plain, MA 02130-3515; Vicam L. P., 313 Pleasant St., Watertown, MA 02172. A superior plus ($^+$) indicates that the culture is a spontaneous variant capable of colony formation on EC agar at 45.5°C.

The number of phenotypic variants in broth cultures capable of growth at 45.5°C was determined by first inoculating tubes containing 9 ml of TSB incubated overnight at 32°C with rotary agitation (200 rpm). Decimal dilutions in TSB were then prepared and 0.1 ml smeared in duplicate onto plates of TSA incubated at 37°C in polyethylene zip bags. Smear plates of EC agar were similarly prepared and incubated at 45.5°C.

Growth curves were obtained by first inoculating 250-ml baffled flasks containing 100 ml of TSB and incubating for 8 to 9 h at 37°C with rotary agitation. Duplicate baffled 250-ml flasks containing 100 ml of either EC broth or EC broth prepared without the addition of bile salts #3 were then inoculated from the 8- to 9-h flasks to yield an initial A_{600} of 0.05 (2.0×10^7 CFU/ml) unless otherwise indicated, followed by incubation at 45.5°C (±0.1°C) in a temperature-controlled incubator chamber with rotary agitation (150 rpm). A_{600} values were then determined every 30 or 60 min and mean values plotted.

EC broth (tryptose, 2.0%; lactose, 0.5%; bile salts #3, 0.15%; K_2HPO_4, 0.4%; KH_2PO_4, 0.15%; and NaCl, 0.5%; pH 6.9) without bile salts #3 was prepared from individual components and adjusted to pH 6.9. The tryptose and bile salts #3 were obtained from Difco and all other components were from Sigma (St. Louis, Mo.).

Isolation of phenotypic variants capable of growth on EC agar at 45.5°C and determination of their numbers in broth cultures. An overnight TSB culture incubated at 32°C was decimally diluted in tubes containing 9.0 ml of TSB. Dilutions (0.1 ml) were then smeared in triplicate onto the surface of EC agar plates and TSA plates. One set of EC plates was incubated at 45.5°C and another at 37°C. The TSA plates were incubated at 37°C. Phenotypic variants capable of developing colonies at 45.5°C on EC agar were then picked and maintained on TSA slants. The number of such tolerant variants per 1×10^9 total CFU in a culture was then calculated from the total number of CFU developing on the TSA plates at 37°C.

Biological assay of bile salts #3 activity. Nutrient agar containing 0.5% glucose (30 ml) was dispensed into screwcapped tubes (25 by 200 mm), steam sterilized, and then the contents of each tube poured into petri dishes (15 by 100 mm). After solidification, the plates were placed at 55°C for 8 h to dry the surface and were then held at 32°C for 72 h to facilitate further moisture loss. The cells from an overnight TSA slant of *B. stearothermophilus* were suspended in 8.0 ml of TSB and 0.1 ml of the cell suspension smeared onto the surface of the nutrient agar containing 0.5% glucose plates. An agar plug (1.5 cm diameter) was removed from the center of each plate with a sterile stainless steel cork borer. The wells were filled with 0.5 ml of sterile EC broth or conditioned EC broth to assess if a detectable quantity of bile salts #3 was removed by cells used for conditioning the EC broth. The plates were incubated at 55°C for 24 h and the diameters (mm) of zones of inhibition recorded. Plates were sprayed with a 0.5% aqueous solution of the cytochrome oxidase substrate *N,N,N′,N′*-tetramethyl *p*-phenylendiamine for photographic enhancement. All such assays were performed in pentuplicate with the mean and standard deviations reported. Student's *t* test was used to determine if the difference in mean zone diameters was statistically significant. The software program Instat (GraphPad Software, Inc., San Diego, Calif.) was used for statistical calculations.

TABLE 2. *Ability of* E. coli *O157:H7 strains and commensulate strain GG to produce turbidity (T) and gas (G) at elevated temperatures in EC broth*[a]

	42.5°C		43.5°C		44.5°C		45.5°C	
Strain	T	G	T	G	T	G	T	G
GG	+	+	+	+	+	+	+	+
E6	+	+	+	+	+	+	+	+
E19	+	+	+	+	+	+	+	+
E66	+	+	+	+	+	+	+	+
E5	+	+	+	+	+	+	−	−
E67	+	+	+	+	+	+	−	−
E68	+	+	+	+	+	+	−	−
E7	−	−	−	−	−	−	−	−
E7$^+$	+	+	+	+	+	+	+	+
C9490	+	+	−	−	−	−	−	−
C9490$^+$	+	+	+	+	+	+	+	+
E18	−	−	−	−	−	−	−	−
E18$^+$	+	+	+	+	+	+	+	+
933	+	+	+	+	−	−	−	−
E4	+	+	+	+	−	−	−	−
E21	+	+	+	+	−	−	−	−
E20	+	+	+	+	−	−	−	−
E59	+	+	+	+	−	−	−	−
E60	+	+	+	+	−	−	−	−
E61	+	+	+	+	−	−	−	−
E62	+	+	+	+	−	−	−	−
E63	+	+	+	+	−	−	−	−
E65	+	+	+	+	−	−	−	−

[a] Inoculum consisted of a total of 1×10^3 CFU added to each tube containing 10 ml of EC broth resulting in an initial CFU density of 1×10^2 ml. A + indicates both tubes were positive for turbidity and gas formation after 48 h. A − indicates both tubes were negative for turbity or gas production. A ± indicates that one tube was positive and one tube was negative for both turbidity and gas formation.

RESULTS

Formation of turbidity and gas at 42.5, 43.5, 44.5, and 45.5°C in EC broth. All EC broth tubes in this study were inoculated with 1×10^2 CFU/ml. *E. coli* strain GG, which is a metabolically typical *E. coli* isolate, produced turbidity and gas at all four incubation temperatures. Among 18 strains of *E. coli* O157:H7 examined, two (E6 and E66) produced turbidity and gas after 48 h of incubation in EC broth at all four elevated temperatures (Table 2). Three strains (E5, E67, and E68) produced turbidity and gas at 42.5, 43,5, and 44.5°C but not at 45.5°C. Ten strains (933, E4, E21, E20, E59, E60, E61, E62, E63, and E65) produced turbidity and gas at 42.5 and 43.5°C but not at 44.5 or 45.5°C. One strain (C9490) produced turbidity and gas only at 42.5°C. Strains E7 and E18 were the most sensitive, with both failing to yield turbidity at all four elevated temperatures in EC broth. Phenotypic variants of strains C9490, E7, and E18 designated C9490$^+$, E7$^+$, and E18$^+$, respectively, produced turbidity and gas at all four temperatures.

Correlation between initial numbers of CFU of *E. coli* O157:H7 strains and production of turbidity and gas in EC broth at 42.5, 43.5, 44.5, and 45.5°C. When the initial density of CFU varied from 1×10^7 to 1×10^0/ml strain E4 developed turbidity and gas at 42.5 and 43.5°C with just 1 CFU/ml after 48 h of incubation (Table 3). In contrast, no less than an initial CFU density of 1×10^3/ml was required for strain E4 to produce turbidity and gas consistently at 44.5 and 45.5°C (Table 3). Strain E18 required no less than 1×10^5 and 1×10^6 CFU/ml to develop turbidity and gas formation at 42.5 and 43.5°C, respectively (Table 4). This strain required no less than 1×10^7 CFU/ml initially to develop turbidity and gas formation at 44.5 and 45.5°C. Phenotypic variant E18$^+$ derived from strain E18 exhibited turbidity and consistent gas formation at all four temperatures with an initial CFU density of 1×10^2/ml but failed to develop at any of the four temperatures

TABLE 3. *Correlation between numbers of initial CFU/ml of* E. coli *O157:H7 strain E4 in EC broth at various incubation temperatures and production of turbidity and gas*[a]

Initial no. of CFU/ml	42.5°C	43.5°C	44.5°C	45.5°C
1×10^7	+	+	+	+
1×10^6	+	+	+	+
1×10^5	+	+	+	+
1×10^4	+	+	+	+
1×10^3	+	+	+	+
1×10^2	+	+	±	±
1×10^1	+	+	−	−
1×10^0	+	+	−	−

[a] See legend to Table 2.

TABLE 4. *Correlation between initial CFU/ml of* E. coli *O157:H7 strains E18 and E18$^+$ and production of turbidity and gas in EC broth at various incubation temperatures*[a]

Initial no. of CFU/ml	42.5°C	43.5°C	44.5°C	45.5°C
Strain E18				
1×10^7	+	+	+	+
1×10^6	+	+	−	−
1×10^5	+	−	−	−
1×10^4	−	−	−	−
1×10^3	−	−	−	−
1×10^2	−	−	−	−
1×10^1	−	−	−	−
1×10^0	−	−	−	−
Strain E18$^+$				
1×10^7	+	+	+	+
1×10^6	+	+	+	+
1×10^5	+	+	+	+
1×10^4	+	+	+	+
1×10^3	+	+	+	+
1×10^2	+	+	+	+
1×10^1	+	±	−	±
1×10^0	−	−	−	−

[a] See legend to Table 2.

TABLE 5. *Frequency of phenotypic variant CFU in TSB capable of growth at 45.5°C on EC agar among various strains of* E. coli *O157:H7*

Strain	37°C (TSA)	37°C (EC agar)	45.5°C (EC agar)	No. of CFU developing at 45.5°C on EC agar among 1×10^9 total CFU[a]
GG	$2.4 \pm 0.3 \times 10^9$	$2.1 \pm 0.1 \times 10^9$	$2.2 \pm 0.4 \times 10^9$	$9.1 \times 10^8 \pm 0.7$
E6	$1.8 \pm 0.0 \times 10^9$	$1.5 \pm 0.2 \times 10^9$	$1.7 \pm 0.4 \times 10^9$	$9.1 \times 10^8 \pm 2.0$
E4	$5.1 \pm 1.9 \times 10^9$	$5.2 \pm 1.8 \times 10^9$	$1.4 \pm 0.4 \times 10^6$	$2.8 \times 10^5 \pm 0.6$
E21	$1.6 \pm 0.0 \times 10^9$	$0.9 \pm 0.3 \times 10^9$	$2.4 \pm 0.4 \times 10^4$	$1.5 \times 10^4 \pm 0.3$
C9490	$2.2 \pm 0.4 \times 10^9$	$0.9 \pm 0.3 \times 10^9$	$6.3 \pm 1.4 \times 10^4$	$3.3 \times 10^4 \pm 0.5$
C9490$^+$	$2.2 \pm 0.4 \times 10^9$	$2.1 \pm 0.2 \times 10^9$	$1.4 \pm 0.3 \times 10^9$	$6.5 \times 10^8 \pm 0.3$
E18	$1.9 \pm 0.1 \times 10^9$	$0.9 \pm 0.2 \times 10^7$	$1.2 \pm 0.2 \times 10^3$	$6.2 \times 10^2 \pm 0.9$
E18$^+$	$3.6 \pm 0.3 \times 10^8$	$3.0 \pm 1.6 \times 10^8$	$1.3 \pm 0.2 \times 10^8$	$3.5 \times 10^8 \pm 0.9$
E7	$2.7 \pm 0.2 \times 10^9$	$6.4 \pm 0.9 \times 10^7$	$8.8 \pm 0.4 \times 10^2$	$3.3 \times 10^2 \pm 0.4$
E7$^+$	$2.1 \pm 0.1 \times 10^9$	$2.1 \pm 0.4 \times 10^9$	$1.5 \pm 0.1 \times 10^9$	$7.1 \times 10^8 \pm 0.2$

[a] Mean of duplicate values derived from two experiments ± one standard deviation from mean.

with 1×10^0 CFU/ml (Table 4). Strain E7 exhibited a spectrum of sensitivity that was similar to that exhibited by strain E18, requiring no less than 1×10^7 initial CFU/ml to develop turbidity and gas at 44.5 and 45.5°C (data not given). Phenotypic variant E7$^+$ derived spontaneously from strain E7 developed turbidity and gas with 1×10^2 initial CFU/ml at all four temperatures but was unable to develop at 44.5 and 45.5°C with 1×10^1 CFU/ml. Strain C9490 exhibited notably less sensitivity and gave rise to turbidity and gas at all four temperatures with 1×10^3 initial CFU/ml but was unable to develop with 1×10^0 initial CFU/ml at all four temperatures. Phenotypic variant C9490$^+$ derived from strain C9490 exhibited turbidity and gas with 1×10^0 initial CFU/ml at all four temperatures (data not given).

Incidence of phenotypic variants capable of growth at 45.5°C on EC agar among varius strains of *E. coli* O157:H7. Cultures of *E. coli* O157:H7 were found to vary widely in their content of phenotypic variants capable of forming colonies on EC agar at 45.5°C (Table 5). Among six wild-type *E. coli* O157:H7 strains examined, strain E6 was the most tolerant, exhibiting the presence of 9.1×10^8 tolerant CFU/1×10^9 total CFU (91%) capable of growth on EC agar at 45.5°C. These results are consistent with those in Table 2. Strains E4 and E21 yielded 2.8×10^5 (0.028%) and 1.5×10^4 (0.0015%) tolerant CFU/1×10^9 total CFU/ml, respectively. Strains C9490 and C9490$^+$ were found to possess 3.3×10^4 (0.0033%) and 6.5×10^8 (65%) tolerant CFU/1×10^9 total CFU/ml, respectively. These results are also consistent with those in Table 2. Strains E18 and E18$^+$ exhibited 6.2×10^2 (0.000062%) and 3.5×10^8 (35%) tolerant CFU/1×10^9 total CFU/ml, respectively (Table 5). Strains E7 and E7$^+$ yielded 3.3×10^2 (0.000033%) and 7.1×10^8 (71%) tolerant CFU/1×10^9 total CFU/ml, respectively. These results are also consistent with those in Table 2 and reflect the high level of sensitivity of the wild-type strains E18 and E7 indicated in Table 2. Strains E18 and E7 were the only strains that exhibited sensitivity to EC agar at 37°C, yielding under these conditions 4.8×10^8 (48%) and 2.4×10^8 (24%) CFU/1×10^9 total CFU, respectively (Table 5).

Growth of strains at 45.5°C on TSA and in TSB. All five strains examined, E18, E4, E21, C9490, and E7 yielded 100% CFU on TSA at 45.5°C when compared to the number of CFU developing at 37°C on TSA (Table 5). Strains were inoculated into 100 ml of TSB at an initial CFU density of 10 CFU/ml and incubated at 45.5°C with rotary agitation in an attempt to duplicate the negative results of Doyle and Schoeni *(1).* All but 1 of the 18 strains (E59) exhibited visual turbidity after 24 h of incubation and all were turbid after 48 h of incubation (data not given). We did observe that when overnight inocula were well into the stationary growth phase the majority of strains failed to yield turbidity after 24 h of incubation at 45.5°C, whereas inocula in the late exponential growth phase yielded turbidity with all but one culture after 24 h of incubation. Cells from all flasks exhibiting turbid growth were confirmed to be *E. coli* by inoculating plates of Levine's EMB agar to exclude the possibility of turbid growth due to contamination.

Growth of *E. coli* 0157:H7 strains in EC broth with and without bile salts #3 at 45.5°C. When wild-type strain E4 was inoculated into EC broth devoid of bile salts at an initial CFU density of 2×10^7/ml ($A_{600} = 0.05$) the generation time obtained at 45.5°C was 30 min (Fig. 1). Strains C9490, E18, and E7 yielded similar results. In contrast, when strain E4 was inoculated into EC broth at an initial CFU density of 2×10^7/ml, growth occurred with a generation time of 75 min (Fig. 1). When, however, strain E4 was inoculated into EC broth at a 10-fold lower CFU density of 2×10^6/ml, growth failed to occur (Fig. 1).

Influence of conditioned medium on ability of strain E4 to grow in EC broth at 45.5°C. Flasks of EC broth were inoculated with strain E4 at an initial CFU density of 2.0×10^7/ml and incubated at 45.5°C for two generations of growth. The cells were then removed by sterile mem-

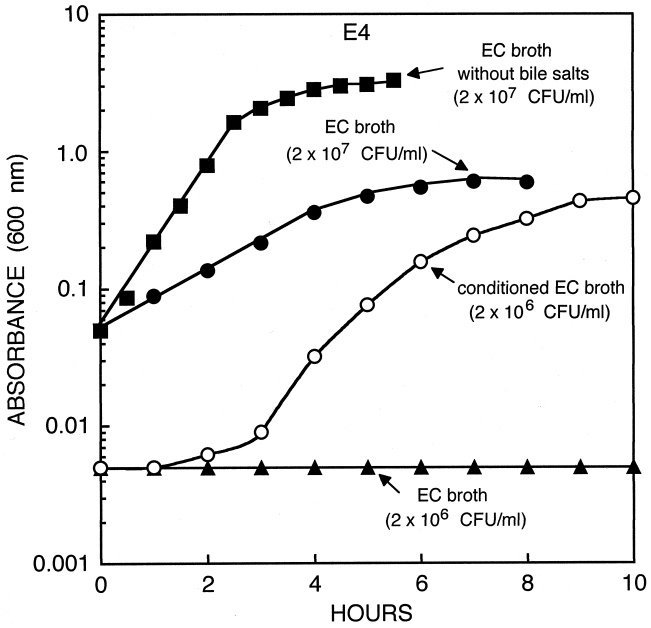

FIGURE 1. *Growth of strain E4 in EC broth, EC broth without bile salts #3, and in conditioned EC broth at 45.5°C. Duplicate 250-ml flasks at 45.5°C containing 100 ml of EC broth or EC broth without bile salts were inoculated with a total of 2 × 10⁹ CFU resulting in 2 × 10⁷ CFU/ml (A_{600} = 0.05) followed by incubation at 45.5°C. Growth in conditioned broth involved duplicate flasks containing 100 ml of EC broth, each of which was inoculated with a total of 2.0 × 10⁹ CFU resulting in 2 × 10⁷ CFU/ml (A_{600} = 0.05), and two generations of growth at 45.5°C were allowed to occur. The culture medium of both flasks was then passed through 0.45-μm porosity membrane filters to remove the majority of CFU followed by passage through 0.25-μm porosity membrane filters. The resulting sterile and conditioned EC broths (100 ml each) were then transferred to duplicate sterile 250-ml baffled flasks, warmed to 45.5°C, and each flask was inoculated with a total of 2 × 10⁸ CFU resulting in 2 × 10⁶ CFU/ml (A_{600} = 0.005) followed by incubation at 45.5°C. A second set of duplicate flasks containing fresh EC broth was also inoculated so as to yield an initial CFU density of 2 × 10⁶/ml and incubated at 45.5°C. Numerical values in this figure indicate the number of CFU/ml at time zero.*

brane filtration to yield conditioned media that were transferred to sterile 250-ml flasks. When these conditioned EC broths were then inoculated to a CFU density of 2.0×10^6/ml with strain E4, growth was initiated after 2 h of incubation at 45.5°C and sustained for over six generations (Fig. 1), reflecting a response to the previous presence of 8.0×10^7 CFU that had been removed.

Assessment of removal of bile salts #3 by cells used for conditioning EC broth. The possibility arose that the process of conditioning EC broth by prior growth of cells at high cell densities might have resulted in the binding of bile salts. When cells were removed, this would have the effect of removing bound bile salts and reducing the inhibition observed with a low density of CFU in the inoculum. We used a microbiological assay to assess the possible removal of bile salts in EC broth by cells of strain E4 used to condition the medium. The quantitative sensitivity of *B. stearothermophilus* to the content of bile salts #3, the only inhibitor present in EC broth, was similar with respect to both fresh EC broth and conditioned EC broth. The difference between the mean diameter of the zone of inhibition with fresh broth (33.0 ± 0.8 mm) and with conditioned EC broth (33.1 ± 0.9 mm) was not statistically significant ($P < 0.05$). These results indicate that the ability of 2×10^6/ml CFU of strain E4 to initiate growth in conditioned EC broth was not due to the removal of bile salts by the notably higher numbers of CFU used to condition the medium.

DISCUSSION

Doyle and Schoeni *(1)* reported the inability of a strain of *E. coli* O157:H7 to increase beyond 5×10^5 CFU/ml (below the range for visible turbidity) starting with about 1×10^3 CFU/ml in TSB during incubation at 44°C to 45°C for 48 h, and that growth failed to occur at 45.5°C. When we used 10 initial CFU/ml in the same medium at 45.5°C, all of the 18 strains yielded heavily turbid growth after 48 h of incubation, reflecting a final CFU density of at least 10^8/ml. It is possible that the strain Doyle and Schoeni *(1)* studied may have been exceptionally sensitive to 45.5°C.

Among the 18 strains we studied, 16 were unable to develop turbidity and gas at 45.5°C during 48 h of incubation in EC broth when inoculated at an initial density of 100 CFU/ml, and 13 were unable to do so at 44.5°C. The report of Raghubeer and Matches *(7)* that *E. coli* O157:H7 is unable to develop turbidity and gas at 44.5°C in EC broth at an initial CFU of 10/ml was based on the examination of a single strain. Our results with the majority of the O157:H7 strains studied are consistent with their observation. Palumbo et al. *(6)* concluded that 1 day of incubation in EC broth at 45°C would be suitable for the detection of gas production by 18 of 23 (78%) of the *E. coli* O157:H7 strains they examined. They used a CFU density of about 10^3/ml, whereas we used a CFU density of 1×10^2 CFU/ml that may account for the lower percentage of O157:H7 strains (6 of 18, 33%) that we examined that were capable of gas production in 48 h. Among seven strains examined, all harbored phenotypic variants at widely varying frequency that were capable of developing turbidity and gas at low CFU densities in EC broth at 45.5°C. The ability to form turbidity and gas at 45.5°C in EC broth was found to be dependent on the presence of low numbers of phenotypic variants tolerant to this medium at 45.5°C that correlated with the initial CFU density.

Our results indicate that the presence of bile salts was notably inhibitory at 45.5°C. When the initial density of CFU was reduced to 2×10^6/ml with strain E4, growth failed to occur in EC broth at 45.5°C unless the medium was conditioned by the previous growth of the organism to a level of 8×10^7 CFU/ml. Removal of the inhibitory bile salts by the cells used for conditioning of the medium was found not to occur.

Our results suggest that an unknown chemical substance is produced in EC broth at 45.5°C by strain E4 during growth with CFU densities above 2×10^7/ml. This chemical agent is capable of stimulating the initiation of growth in EC broth at 45.5°C with an initial CFU density of 2×10^6/ml and therefore functionally resembles the au-

toinducer of luminescence produced by *Vibrio fischeri (3, 4)* that is dependent on cell density. Such cell density-dependent phenomena expressed by microbial populations are referred to as quorum-sensing *(4).* With *V. fischeri* the cells produce a diffusible compound termed autoinducer that accumulates in the surrounding environment during growth. At low cell densities this substance is at concentrations below a specific threshold level, while at high cell densities this substance reaches the critical threshold concentration for activation of luminescence genes *(3–5).* The present study is the first report of *E. coli* exhibiting a phenomenon resembling a quorum response. The phenomenon may be of significance in terms of the ability of the organism to develop in microenvironments, under marginally adverse conditions, where diffusion of the critically required metabolite is limited.

ACKNOWLEDGMENTS

This material is based upon work supported by the Cooperative State Research, Extension, Education Service, U.S. Department of Agriculture, Massachusetts Agricultural Experiment Station, under project no. 744 and is report no. 3237.

REFERENCES

1. Doyle, M. P., and J. L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855–856.
2. Eberhard, A. 1972. Inhibition and activation of bacterial luciferase synthesis. J. Bacteriol. 109:1101–1105.
3. Eberhard, A., A. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson, and N. J. Oppenheimer. 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry 20: 2444–2449.
4. Fuqua, W. C., S. C. Winans, and E. P. Greenberg. 1994. Minireview. Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. J. Bacteriol. 176:269–275.
5. Nealson, K. H. 1977. Autoinduction of bacterial luciferase: occurrence, mechanism and significance. Arch. Microbiol. 112:73–79.
6. Palumbo, S. A., J. E. Call, F. J. Schultz, and A. C. Williams. 1995. Minimum and maximum temperatures for growth and verotoxin production by hemorrhagic strains of *Escherichia coli.* J. Food Prot. 58: 352–356.
7. Raghubeer, E. V., and J. R. Matches. 1990. Temperature range for growth of *Escherichia coli* serotype O157:H7 and selected coliforms in *E. coli* medium. J. Clin. Microbiol. 28:803–805.