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The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7

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The [6] effects of initial pH, sodium chloride content, and incubation temperature on the aerobic and anaerobic growth kinetics of a three strain mixture of Escherichia coli *O157:H7 were evaluated using brain heart infusion broth. The three variables interacted to affect growth, with the primary effects being noted in relation to generation times (GTs) and lag phase durations (LPDs). The maximum population densities (MPDs) achieved by the cultures were largely independent of the three variables; however, there was a general depression of MPDs by 0·5–1·0 log cycles when the cultures were incubated anaerobically. Under otherwise optimal conditions, GTs and LPDs were largely unaffected by initial pH at values ⩾ 5·5. Initial pH had a greater effect when the NaCl content was elevated. Increasing NaCl levels decreased the growth rate of the organism, with the effect being greater if the other variables were also non-optimal. In general, the effect of temperature could be adequately described by the Ratkowsky square root function; however, there was a general depression of optimal growth temperatures and an increase in the differential between T_{min} and actual temperature that did not support growth as other variables became non-optimal. Comparison of the current data with previous reports suggest that the growth kinetics of* E. coli *O157:H7 are similar to those for non-pathogenic strains.*

Introduction

The identification of *Escherichia coli* O157:H7 as a causative agent of haemorrhagic colitis and haemolytic uremic syndrome has quickly led it to being recognized as a foodborne pathogen of primary concern. During the past several years there has been substantial research pertaining to the detection of the micro-organism and characterization of factors contributing to its virulence. However, little information has been provided concerning factors that would affect its growth in foods; the organism has been assumed to behave similarly to non-pathogenic strains. This is particularly true in relation to quantitative growth kinetics data that could be used to estimate the growth of O157:H7 strains in food systems. Over the past several years, our laboratory has been involved in the development of mathe-

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matical models that describe the effects of multiple cultural parameters on the growth kinetics of foodborne pathogens. As part of that effort, data were gathered on the effects and interactions of incubation temperature, initial pH, sodium chloride content, and oxygen availability on the growth of *E. coli* O157:H7. The purpose of this manuscript is to present those data and to describe some of the qualitative relationships observed.

Materials and Methods

Micro-organisms

Three *E. coli* O157:H7 strains, 933, 45753-35, and A9218-C1, were employed throughout the study. Working stock cultures were maintained in the Brain Heart Infusion broth (BHI) stored at 4°C and transferred monthly.

Experimental data

A fractional factorial design was employed to assess the effects of incubation temperature (5°C–42°C), initial pH (4·5–8·5), sodium chloride 0·5–5·0% (w/v), and oxygen availability (aerobic vs anaerobic). The number of replicate cultures examined for each variable combination tested is indicated in Tables 1 and 2.

Culture techniques

Starter cultures were prepared by transferring 100 μl of the working stock cultures to individual 250 ml Erlenmeyer flasks containing 50 ml of BHI, and incubating the flasks for 18–20 h at 37°C on a rotary shaker (150 rpm). A 1·0 ml portion of each starter culture was transferred to a tube containing 7·0 ml of 0·1% peptone water to yield an equal mixture of stains at an overall level of approximately 2×10^9 cfu ml^{-1}. Additional dilutions were made to achieve a level of approximately 2×10^5 cfu ml^{-1}.

Test cultures were prepared and sampled using a modification of the techniques of Buchanan et al. (1989). Briefly, BHI was rehydrated, supplemented with NaCl as required, brought up to volume, and the pH adjusted using 10 N HCl or 50% KOH. The medium was dispensed in 50 ml portions to either 250 ml Erlenmeyer flasks (aerobic cultures) or trypsinizing flasks with a rubber septum inserted in the sidearm sampling port. The flasks were capped with a foam plug (aerobic) or a screw cap (anaerobic), and sterilized by autoclaving for 15 min at 121°C.

After cooling, each flask received 0·5 ml of the mixture of the three tester strains to achieve an inoculum of approximately 10^3 cfu ml^{-1}. The trypsinizing flasks were then flushed with sterile N_2 for 10 min and sealed with a rubber stopper. All flasks were subsequently incubated at the appropriate temperatures on rotary shakers (150 rpm). Periodically, 2·5 ml samples were removed, diluted appropriately using 0·1% peptone water, and surface plated in duplicate on Brain Heart Infusion Agar (BHIA) using a Spiral Plater. All plates were incubated at 37°C for 20–24 h and then enumerated.

Curve fitting

Growth curves were generated by fitting the Gompertz function to the data as previously described (Gibson et al. (1987); Buchanan et al. (1989)). The Gompertz function parameters were then used to calculate lag phase duration (LPDs), generation times (GTs), and maximum population densities (MPDs). The estimated time to achieve an increase in population density from 10^2 to 10^5 cfu ml^{-1} was found to be a convenient method measuring the integral impact of the variables on LPD and GT. This value was determined by rearranging the Gompertz equation to solve for time, assuming an A value of 2·0.

Results

A total of 184 aerobic (Table 1) and 144 anaerobic (Table 2) cultures were generated, representing 75 and 70 variable combinations, respectively. Comparison of LPDs, GTs and estimated time to achieve a three log cycle increase in population density (10^2 to 10^2 cfu ml^{-1}) suggested that aerobic conditions tended to enhance growth. However, the differences between aerobic and anaerobic cultures tended to be minimal, indicating that the organism is well adapted to anaerobic conditions. A differential in the MPDs of aerobic and anaerobic

Table 1. Effect of incubation temperature, initial pH, and sodium chloride concentration on the aerobic growth kinetics of *Escherichia coli* O157:H7[a].

Temp.	pH	NaCl	*n*	EGR	GT	LPD	MPD	Time to 3-log increase (hour)[b]
5	5·50	5	1	0·000	No growth	—	—	—
5	6·50	5	3	0·000	No growth	—	—	—
5	7·50	5	1	0·000	No growth	—	—	—
8	5·50	5	1	0·000	No growth	—	—	—
8	6·00	20	1	0·000	No growth	—	—	—
8	6·50	5	1	0·000	No growth	—	—	—
8	7·00	20	1	0·000	No growth	—	—	—
8	7·50	5	1	0·000	No growth	—	—	—
8	8·50	5	1	0·000	No growth	—	—	—
10	4·50	5	3	0·000	No growth	—	—	—
10	5·50	5	3	0·066(0·002)	4·6(0·2)[c]	38·4(0·4)	10·0(0·1)	121·6
10	5·50	20	3	0·000	No growth	—	—	—
10	6·50	5	3	0·049(0·001)	6·2(0·1)	44·2(2·4)	9·8(0·1)	147·2
12	5·50	5	1	0·059	5·1	24·7	10·1	100·4
12	5·50	20	1	0·048	6·3	48·2	9·4	152·8
12	6·00	5	1	0·060	5·0	21·9	9·8	91·2
12	6·00	20	1	0·055	5·5	30·2	9·5	110·7
12	6·50	20	1	0·058	5·2	26·9	9·7	102·0
12	6·50	35	1	0·015	19·6	173·1	8·0	489·5
12	6·50	50	1	0·000	No growth	—	—	—
12	7·00	20	1	0·057	5·3	27·1	9·9	105·1
12	7·50	5	1	0·060	5·0	71·9	9·5	189·8
12	7·50	50	1	0·000	No growth	—	—	—
12	8·00	20	1	0·107	2·8	79·3	9·9	186·0
12	8·50	5	1	0·059	5·1	46·6	9·7	141·1
19	4·50	5	3	0·120(0·006)	2·5(0·1)	17·5(2·0)	10·1(0·1)	59·9
19	4·50	20	3	0·26(0·001)	11·8(0·5)	83·5(11·7)	9·8(0·1)	279·1
19	4·50	35	3	0·000	No growth	—	—	—
19	4·50	50	3	0·000	No growth	—	—	—
19	5·00	20	1	0·117	2·6	10·4	9·9	45·7
19	5·50	5	3	0·256(0·031)	1·2(0·2)	12·2(1·4)	10·2(0·3)	36·4
19	5·50	50	2	0·090(0·001)	3·4(0·1)	37·5(0·2)	9·2(0·1)	104·5
19	6·00	20	1	0·252	0·3	17·2	9·4	36·8
19	6·50	5	3	0·247(0·005)	1·2(0·1)	9·7(0·2)	9·7(0·1)	30·8
19	6·50	20	3	0·223(0·043)	1·4(0·2)	12·9(2·1)	10·3(0·6)	40·0
19	6·50	35	2	0·123(0·005)	2·5(0·1)	16·0(0·4)	9·5(0·1)	54·5
19	6·50	50	3	0·057(0·002)	5·3(0·2)	21·7(5·3)	9·6(0·1)	92·7
19	7·00	20	1	0·269	1·1	15·8	9·7	42·0
19	7·00	50	15	0·143(0·014)	2·1(0·2)	34·2(2·5)	9·4(0·2)	87·4
19	7·20	5	6	0·249(0·019)	1·2(0·1)	10·6(1·6)	9·7(0·1)	32·5
19	7·50	5	3	0·261(0·016)	1·2(0·1)	9·2(0·9)	9·6(0·1)	29·1
19	7·50	50	3	0·065(0·006)	4·7(0·5)	105·7(4·2)	8·7(0·2)	249·3
19	8·00	20	1	0·117	2·6	17·1	9·9	59·1
28	4·50	5	2	0·454(0·023)	0·7(0·1)	5·0(0·1)	9·3(0·1)	16·0
28	4·50	20	3	0·271(0·006)	1·1(0·1)	7·0(0·5)	9·5(0·1)	24·1
28	4·50	35	3	0·030(0·004)	10·5(1·7)	5·2(4·2)	6·5(0·3)	66·1
28	4·50	50	a	0·133	2·3	41·9	6·7	96·4
28	5·50	5	1	0·573	0·5	1·8	9·9	8·7

(continued)

Table 1. Effect of incubation temperature, initial pH, and sodium chloride concentration on the aerobic growth kinetics of *Escherichia coli* O157:H7[a]. — *continued*

Temp.	pH	NaCl	*n*	EGR	GT	LPD	MPD	Time to 3-log increase (hour)[b]
28	5·50	50	3	0·305(0·082)	1·1(0·3)	42·5(2·3)	8·7(0·1)	93·8
28	6·50	5	3	0·548(0·014)	0·6(0·1)	3·0(0·1)	9·7(0·1)	11·0
28	6·50	20	3	0·530(0·080)	0·6(0·1)	4·0(0·2)	9·4(0·1)	13·2
28	6·50	35	3	0·414(0·024)	0·7(0·1)	7·6(0·9)	9·5(0·1)	22·0
28	6·50	50	3	0·302(0·008)	1·0(0·1)	16·1(0·5)	9·7(0·1)	41·7
28	7·00	35	1	0·440	0·7	4·8	10·0	16·2
28	7·20	5	15	0·505(0·067)	0·6(0·1)	2·4(0·5)	9·5(0·1)	10·3
28	7·50	5	3	0·537(0·004)	0·6(0·0)	2·8(0·2)	9·4(0·1)	10·7
28	7·50	50	3	0·334(0·009)	0·9(0·1)	18·2(0·3)	9·5(0·1)	44·5
28	8·00	20	1	0·488	0·6	2·2	9·6	10·1
28	8·50	5	3	0·450(0·007)	0·7(0·1)	2·9(0·2)	9·5(0·1)	11·9
28	8·50	35	1	0·247	1·2	7·6	9·4	26·2
37	4·50	5	3	0·284(0·201)	1·1(1·5)	3·8(5·4)	8·8(0·1)	16·4
37	4·50	50	3	0·000	No growth	—	—	—
37	5·50	5	3	0·755(0·063)	0·4(0·1)	1·0(0·4)	9·3(0·4)	5·7
37	5·50	50	4	0·233(0·055)	1·4(0·3)	27·7(9·3)	8·2(0·8)	65·7
37	6·50	5	3	1·001(0·005)	0·3(0·0)	1·4(0·0)	9·6(0·0)	5·6
37	6·50	50	3	0·368(0·030)	0·8(0·1)	10·5(4·0)	9·1(0·1)	28·0
37	7·50	5	3	1·125(0·068)	0·3(0·1)	1·9(0·3)	9·6(0·1)	6·3
37	7·50	50	3	0·457(0·067)	0·7(0·1)	7·2(0·9)	9·3(0·1)	20·4
37	8·50	5	2	0·891(0·070)	0·3(0·1)	2·2(0·4)	9·1(0·1)	7·4
37	8·50	50	2	0·487(0·091)	0·6(0·1)	25·5(0·2)	9·1(0·1)	56·5
42	4·50	5	2	0·423(0·009)	0·7(0·1)	5·2(0·1)	8·1(0·1)	15·7
42	6·50	5	3	1·045(0·033)	0·3(0·1)	1·5(0·3)	9·5(0·1)	5·7
42	6·50	50	1	0·402	0·8	26·3	9·2	59·2
42	8·50	5	2	1·088(0·0000)	0·3(0·0)	1·8(0·1)	9·0(0·1)	6·0
42	8·50	50	1	0·000	No growth	—	—	—

Total: 184

[a] Air flushed atmosphere.
[b] Calculated time for cultures to increase from 100 to 100 000 cfu ml^{-1}.
[c] Values represent means (± standard deviations).
Abbreviations: Temp = Degrees Celsius; NaCl = g l^{-1}; EGR = Exponential Growth Rate (log (cfu ml^{-1})/h); GT = Generation Time (h); LPD = Lag Phase Duration (h); MPD = Maximum Population Density (Log (cfu ml^{-1}), *n* = Number of replicates.

cultures was noted, with the MPD achieved by aerobic cultures generally 0·5–1·0 log cycles higher.

The MPD was largely independent of the three cultural variables. If the organism grew, it typically achieved a MPD between 10^8 and 10^{10} cfu ml^{-1}. Only when the cultural conditions were bordering on those that prevented any growth was any large suppression of MPD noted. This was most apparent when two or more of the three variables were non-optimal. For example, the mean MPD attained by the 28°C/pH 4·5/0·5% NaCl aerobic cultures was 9·34, and that for the 28°C/pH 6·5/5·0 NaCl cultures was 9·69. However, the 28°C/pH 4·5/3·5 NaCl, where both pH and NaCl were limiting, only attained a MPD of 6·53.

Table 2. Effect of incubation temperature, initial pH, and sodium chloride concentration on the anaerobic growth kinetics of *Eschericia coli* O157:H7[a].

Temp.	pH	NaCl	*n*	EGR	GT	LPD	MPD	Time to 3-log increase (hour)[b]
5	5·50	5	1	0·000	No growth	—	—	—
5	6·50	5	3	0·000	No growth	—	—	—
5	7·50	5	1	0·000	No growth	—	—	—
8	5·50	5	1	0·000	No growth	—	—	—
8	6·00	20	1	0·000	No growth	—	—	—
8	6·50	5	1	0·000	No growth	—	—	—
8	7·00	20	1	0·000	No growth	—	—	—
8	7·50	5	1	0·000	No growth	—	—	—
8	8·50	5	1	0·000	No growth	—	—	—
10	4·50	5	3	0·000	No growth	—	—	—
10	5·50	5	3	0·031(0·001)	9·8(0·2)[c]	39·5(6·2)	9·6(0·1)	169·0
10	5·50	20	3	0·000	No growth	—	—	—
10	6·50	5	3	0·045(0·004)	6·8(0·6)	48·7(2·0)	9·2(0·2)	157·6
12	5·50	5	1	0·067	4·5	25·9	9·0	90·2
12	5·50	20	1	0·049	6·1	38·3	8·8	127·1
12	6·00	5	1	0·066	4·6	22·8	9·2	86·0
12	6·00	20	1	0·077	3·9	43·8	8·8	119·6
12	6·50	20	1	0·059	5·1	21·3	9·0	86·1
12	6·50	35	1	0·018	17·2	296·3	6·4	680·2
12	6·50	50	1	0·000	No growth	—	—	—
12	7·00	20	1	0·065	4·7	34·0	9·4	110·2
12	7·50	5	1	0·052	5·8	35·2	9·8	126·1
12	7·50	50	1	0·000	No growth	—	—	—
12	8·00	20	1	0·040	7·5	58·8	9·9	189·8
12	8·50	5	1	0·037	8·2	49·5	9·0	168·9
19	4·50	5	3	0·074(0·004)	4·1(0·2)	17·7(1·5)	7·9(0·4)	64·3
19	4·50	20	3	0·036(0·009)	9·0(2·6)	74·9(16·6)	6·8(0·6)	201·1
19	4·50	35	3	0·000	No growth	—	—	—
19	4·50	50	3	0·000	No growth	—	—	—
19	5·00	20	1	0·125	2·4	16·3	9·1	53·3
19	5·50	5	3	0·243(0·008)	1·2(0·1)	8·8(0·6)	9·1(0·6)	28·3
19	5·50	50	2	0·097(0·001)	3·1(0·1)	41·9(1·3)	8·8(0·1)	109·4
19	6·00	20	1	0·178	1·7	11·8	9·2	38·4
19	6·50	5	3	0·223(0·003)	1·4(0·1)	6·4(0·6)	9·1(0·1)	24·5
19	6·50	20	3	0·240(0·018)	1·3(0·1)	14·2(1·2)	9·4(0·2)	39·9
19	6·50	35	2	0·123(0·005)	2·5(0·1)	16·0(0·4)	9·5(0·1)	54·5
19	6·50	50	3	0·046(0·000)	6·5(0·1)	15·2(3·0)	8·7(0·1)	83·3
19	7·00	20	1	0·254	1·2	17·2	9·3	44·9
19	7·50	5	3	0·236(0·006)	1·3(0·1)	5·7(0·6)	9·3(0·2)	22·8
19	7·50	50	3	0·025(0·005)	12·3(2·4)	57·5(6·4)	8·3(0·2)	208·5
19	8·00	20	1	0·116	2·6	20·8	9·7	65·9
28	4·50	5	2	0·323(0·001)	0·9(0·0)	7·3(1·2)	8·1(0·1)	21·5
28	4·50	20	3	0·619(0·196)	0·5(0·2)	17·2(7·7)	8·4(0·1)	38·6
28	4·50	35	3	0·060(0·011)	5·2(1·0)	12·1(9·0)	7·4(0·4)	57·8
28	4·50	50	1	0·041	7·4	57·5	7·2	161·1
28	5·50	5	1	0·696	0·4	2·8	9·3	9·3
28	5·50	50	3	0·122(0·018)	2·5(0·3)	22·6(3·3)	8·0(0·1)	63·5
28	6·50	5	3	0·514(0·030)	0·6(0·1)	2·6(0·3)	9·0(0·1)	10·2

(continued)

Table 2. Effect of incubation temperature, initial pH, and sodium chloride concentration on the anaerobic growth kinetics of *Escherichia coli* O157:H7[a]. — *continued*

Temp.	pH	NaCl	*n*	EGR	GT	LPD	MPD	Time to 3-log increase (hour)[b]
28	6·50	20	3	0·441(0·067)	0·7(0·1)	3·2(0·2)	8·9(0·1)	12·3
28	6·50	35	3	0·424(0·013)	0·7(0·1)	6·0(0·2)	9·0(0·1)	18·1
28	6·50	50	3	0·393(0·040)	0·9(0·1)	16·9(0·6)	9·1(0·1)	40·5
28	7·00	35	1	0·535	0·6	5·6	9·4	16·4
28	7·50	5	3	0·497(0·020)	0·6(0·1)	2·3(0·2)	9·2(0·2)	10·0
28	7·50	50	3	0·406(0·029)	0·8(0·1)	18·3(0·6)	8·9(0·5)	43·0
28	8·00	20	1	0·410	0·7	2·9	9·4	12·4
28	8·50	5	3	0·446(0·008)	0·7(0·1)	2·8(0·1)	9·4(0·2)	11·6
28	8·50	35	1	0·424	0·6	18·9	9·7	43·7
37	4·50	5	3	0·544(0·199)	0·6(0·2)	6·6(1·4)	7·2(0·1)	16·9
37	4·50	50	3	0·019[d]	15·5	46·4	5·8	197·1
37	5·50	5	3	0·855(0·072)	0·4(0·1)	2·4(0·2)	9·2(0·1)	8·0
37	5·50	50	2	0·318(0·024)	1·0(0·1)	15·7(0·8)	8·3(0·1)	38·7
37	6·50	5	3	1·014(0·012)	0·3(0·0)	1·7(0·1)	8·9(0·2)	5·8
37	6·50	50	4	0·248(0·058)	1·3(0·3)	12·7(03·8)	8·7(0·2)	35·9
37	7·50	5	3	1·042(0·444)	0·4(0·2)	3·0(1·2)	9·2(0·2)	9·4
37	7·50	50	4	0·354(0·074)	0·9(0·2)	11·8(4·0)	8·6(0·3)	30·7
37	8·50	5	2	0·596(0·002)	0·7(0·0)	7·3(0·2)	8·8(0·1)	20·7
42	4·50	5	2	0·635(0·049)	0·6(0·1)	9·8(2·8)	8·5(0·2)	24·5
42	6·50	5	3	1·213(0·018)	0·3(0·0)	1·8(0·1)	8·3(0·1)	5·6
42	8·50	5	2	0·803(0·089)	0·5(0·1)	7·7(0·3)	8·3(0·1)	18·8

Total: 144

[a] Nitrogen flushed atmosphere.
[b] Calculated time for cultures to increase from 100 to 100 000 cfu ml^{-1}.
[c] Values represent means (± standard deviations).
[d] Only one of three cultures grew. Values are those on that culture.
Abbreviations: Temp = Degrees Celsius; NaCl = g l^{-1}; EGR = Exponential Growth Rate (log (cfu ml^{-1})/h); GT = Generation Time (h); LPD = Lag Phase Duration (h); MPD = Maximum Population Density (log (cfu ml^{-1})); *n* = Number of replicates.

Effect of initial pH

Initial pH influenced both LPD and GT. However, the effects were relatively small at pH values ≥ 5·5, particularly at low sodium chloride concentrations. For example, Fig. 1 depicts the effects of initial pH on the estimated time for aerobic cultures containing 0·5% NaCl and incubated at 19, 28, and 37°C to increase from 10^2 to 10^5 cfu ml^{-1}. Using the growth kinetic data in conjunction with the Gompertz function to determine the time to achieve a specified population density increase proved to be an effective means of depicting combined effects on LPD and GT. It is apparent that only pH 4·5 had a large impact on the growth. This was true at all three temperatures. It is interesting to note that at all three temperatures, decreasing the initial pH from 5·5 to 4·5 resulted in an approximate doubling of the estimated time to reach 10^5 cfu ml^{-1}.

Sodium chloride content had a substantial influence on the effect of initial pH, as exemplified by Fig. 2. This figure

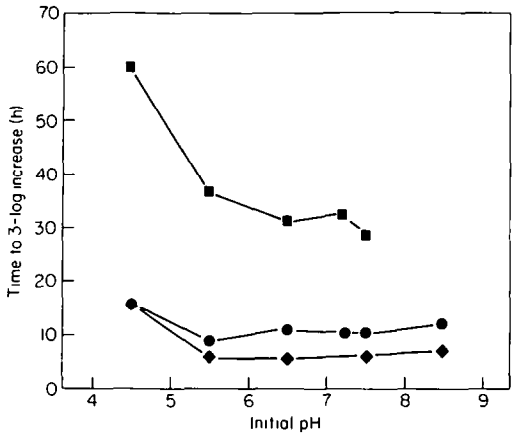

Fig. 1. Effect of initial pH on the growth of aerobic cultures of *Escherichia coli* O157:H7 incubated at three temperatures. Sodium chloride level of all cultures was 0·5%. ■ 19°C; ● 28°C; ♦ 37°C.

depicts the relative impact of initial pH on the growth of 37°C aerobic cultures containing high and low levels of sodium chloride. At the lower concentration (0·5%), the effect of initial pH was small over the entire pH range. When the sodium chloride level was increased to 5·0%, the time to attain a population density of 10^5 cfu ml^{-1} was only slightly greater for initial pH values 6·5 and 7·5. However, increasing or decreasing the pH from these optima resulted in substantial decrease in growth rate. These data suggest that there is a significant interaction between initial pH and sodium chloride content.

Effect of sodium chloride

The effect of sodium chloride concentration on the growth of *E. coli* O157:H7 was affected by both initial pH and incubation temperature. For example, Fig. 3 depicts the effect of sodium chloride on the time to reach 10^5 cfu ml^{-1} for aerobic cultures that had initial pH values of 6·5 and 4·5 and were incubated at 19

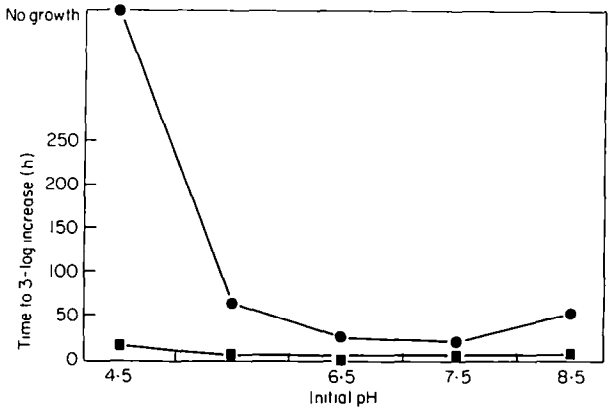

Fig. 2. Effect of high and low sodium chloride levels on the response of aerobic cultures of *Escherichia coli* O157:H7 to varying initial pH levels. Incubation temperature was 37°C. ■ 0·5% NaCl; ● 5·0% NaCl.

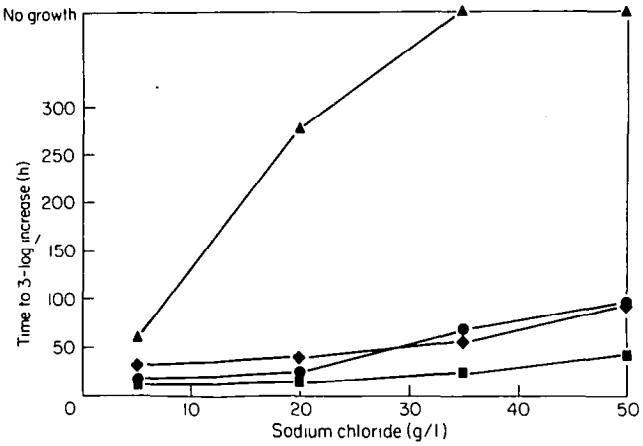

Fig. 3. Effect of sodium chloride concentration on the time required for aerobic cultures of *Escherichia coli* O157:H7 to achieve a 3-log cycle increase. ■ 28°C/pH 4·5; ● 28°C/pH 6·5; ◆ 19°C/pH 6·5; ▲ 19°C/pH 4·5.

and 28°C. At 28°C, growth rate was linearly related to sodium chloride content, and the interaction between pH and sodium chloride appeared small. A similar linear response was observed at 19°C for the pH 6·5 cultures. However, the combination of a non-optimal initial pH and incubation temperature greatly increased the inhibitory activity of sodium chloride. This suggests that the effect of sodium chloride is dependent on a three-way interaction with the other two variables.

Effect of incubation temperature

Under optimal conditions (0·5% NaCl; pH 6·5–7·5), *E. coli* O157:H7 was capable of dividing every 20–30 min when the incubation temperature was 37°–42°C. Decreasing the temperature increased the GT and LPD. Growth was observed both aerobically and anaerobically at 10°C, but not at 8°C. An example of the interaction between incubation temperature and initial pH for cultures having low (0·5%) levels of sodium chloride is presented in Fig. 4 which depicts the time for aerobic cultures to attain a 3-log increase in population density. The response of the organism to incubation temperature was similar except in the pH 4·5 cultures. The combined effects of non-optimal pH and incubation temperature resulted in the micro-organism being unable to grow at ≤12°C. The organism's growth rate at low temperatures also appeared to be somewhat depressed at alkaline pH's. However, additional trials are needed to confirm that preliminary observation.

Two examples of the effect of sodium chloride concentration on the response of *E. coli* O157:H7 to incubation temperature are depicted in Fig. 5. At optimal temperatures, elevated levels of sodium chloride had a relatively small effect on the time achieve a 3-log increase. However, the combined effect of low incubation temperature and high sodium chloride levels strongly suppressed growth rates and raised the minimum temperature that would support growth. Again, the organism appeared to be less capable of handling adverse conditions at pH 7·5.

The square roots of the reciprocals of the time to a 3-log cycle increase for three pH/NaCl combinations, 6·5/0·5%, 6·5/5·0%, and 4·5/0·5%, were plotted against incubation temperature to provide examples of how well the relation-

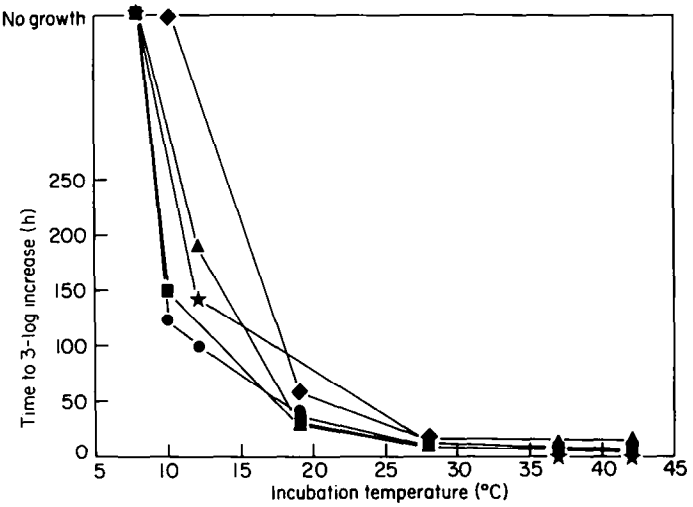

Fig. 4. Effect of incubation temperature on the growth of aerobic cultures of *Escherichia coli* O157:H7 having different initial pH values. The sodium chloride content of all cultures was 0·5%. ♦ initial pH 4·5; ● pH 5·5; ■ pH 6·5; ▲ pH 7·5; ★ pH 8·5.

ship described by Ratkowsky et al. (1982, 1983) fit the current data (Fig. 6). The 6·5/0·5% cultures fit the typical response described by Ratkowsky et al. (1982), with points only deviating from a linear response at temperatures > T_{opt} or approaching the notational minimum temperature, T_{min} (where the linear regression crosses the X-axis). It should be noted that too few incubation temperatures were performed to get an accurate measure to T_{min} so it was not possible to assess the impact on the cultural variables on that parameter. When the sodium chloride content was increased to 5·0%, a similar relationship between incubation temperature and the square root function was observed except the differentials between T_{min} and the actual temperatures that did not support growth were greater. A substantially reduced temperature range over which

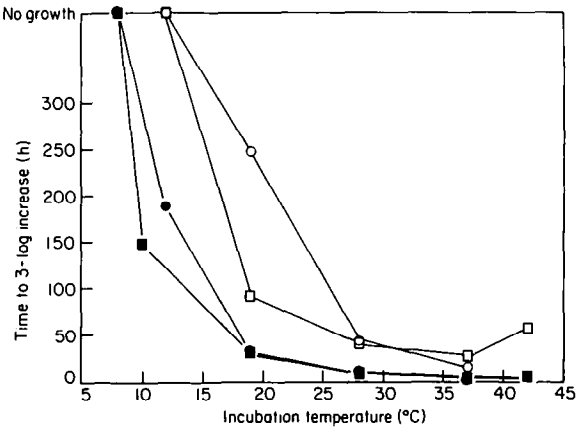

Fig. 5. Effect of incubation temperature on the growth of aerobic cultures of *Eschericia coli* O157:H7 having high and low levels of sodium chloride. ■ pH 6·5/0·5% NaCl; □ pH 6·5/5·0% NaCl; ● pH 7·5/0·5% NaCl; ○ pH 7·5/5·0% NaCl.

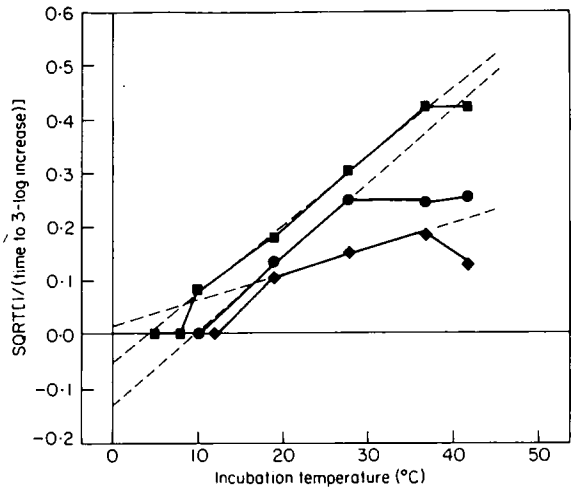

Fig. 6. Examples of the effectiveness of using the square root relationship of Ratkowsky et al. (1982, 1983) to describe the effect of incubation temperature on the growth of *Escherichia coli* O157:H7. ■ pH 6·5/0·5% NaCl; ♦ pH 6·5/5·0% NaCl; ● pH 4·5/0·5 NaCl.

the linear relationship was maintained was observed with the 4·5/0·5% cultures. There appears to have been a reduction in the T_{opt} when the cultures were grown under adverse conditions.

Discussion

The growth of *E. coli* O157:H7 was affected by incubation temperature, initial pH, and sodium chloride concentration, with a strong indication that the three variables are interactive. Based on qualitative data, other investigators have concluded that the growth of enteropathogenic *E. coli* is dependent on the interaction of storage temperature, pH, sodium chloride (water activity), sodium nitrite and phosphates (Gibson and Roberts 1986, Hughes and McDermott 1989). The micro-organism responded in a similar manner under aerobic and anaerobic conditions, indicating that the organism is well adapted for anaerobic growth. However, the consistent reduction in the MPD achieved by anaerobic cultures does indicate a physiological advantage associated with oxygen availability. The primary effects of the three variables were alterations in LPD and GT. Except for extremes of cultural conditions, if the organism initiated growth, its MPD was independent of the three variables.

The organism's general response was different for each of the three variables. Particularly when other variables are non-restrictive, the effect of incubation temperature appears to be reasonably described by the square root function. Increases in NaCl over the range from 0·5–5·0% produced approximate linear changes in LPD and GT unless multiple variables were limiting. Initial pH had relatively little impact on growth kinetics until the pH was < 5·5. In general, the impact of changing cultural conditions was similar for LPD and GT. However, there were enough examples of differences in relative responses that it is apparent that the two phases should be considered separate physiological events and that the impact of cultural conditions on these growth kinetics should be determined independently. Expressing the data as the time to achieve a specified increase in

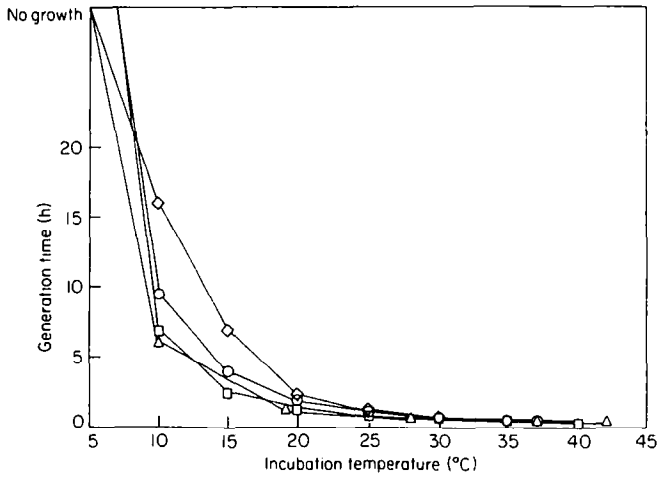

Fig. 7. Comparison of the effect of incubation temperature on generation times observed for the pH 6·5/0·5% NaCl/aerobic *Escherichia coli* O157:H7 cultures in the current study (Δ) and those reported for the growth of *E. coli* type 1 on muscle slices (○) (Shaw and Nicol 1969), *E. coli* SF in ground mutton (□) (Smith 1985), and *E. coli* K12 in brain heart infusion broth (◇) (Gill and Phillips 1985).

population density was an effective means of combining the effects on LPD and GT.

Direct comparison of the growth kinetics of *E. coli* O157:H7 observed in the current study with reported values for other *E. coli* isolates is difficult due to differing substrates and cultural conditions. However, such comparisons do suggest that O157:H7 grows at approximately the same rate as non-pathogenic isolates. For example, Fig. 7 depicts a comparison of the effect of incubation temperature on generation times observed for the pH 6·5/0·5% NaCL/aerobic cultures in the current study and those reported for the growth of non-pathogenic *E. coli* isolates in BHI (Gill and Phillips 1985), in ground mutton (Smith 1985), and on meat slices (Shaw and Nicol 1969). In the absence of specific data related to the enterohaemorrhagic strain, it appears that kinetic data for non-pathogenic strains could be reasonably substituted to provide an estimate of the pathogen's behavior.

The data is currently being analyzed to develop multi-variable mathematical models similar to those that have been derived for *Salmonella* (Gibson et al. 1988), *Listeria monocytogenes* (Buchanan and Phillips 1990), and *Aeromonas hydrophila* (Palumbo et al. 1991, 1992). Once complete, these models should allow a more detailed assessment of the growth kinetics of *E. coli* O157:H7.

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