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Optimal growth temperature of O157 and non-O157 *Escherichia coli* strains

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Aims: There are several biological characteristics that differ between *Escherichia coli* O157:H7, a dangerous food-borne pathogen, and the other serotypes of *E. coli*.

Methods and Results: The optimal growth temperatures (T_{opt}) were determined for 32 *E. coli* strains, whether each strain belonged to the O157 serotype or not. The mean values of T_{opt} for the O157 and non-O157 groups were 40·2 and 41·2°C, respectively.

Significance and Impact of the Study: This difference is statistically significant ($P = 0{\cdot}0002$) but has no biological implication.

INTRODUCTION

The serotype O157:H7 of the verotoxin-producing *Escherichia coli* (VTEC) was first recognized as a serious human pathogen in 1982. This organism was implicated as the agent causing haemorrhagic colitis, and is also associated with haemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Most outbreaks are food- or water-related and have been linked in particular to the consumption of undercooked ground beef. *Escherichia coli* O157:H7 is actually a food-borne pathogen of primary public health concern in North America, Europe and Japan, whose dairy cattle have been identified as the reservoir (Doyle 1991; Coia 1998).

Given the severity of the illnesses associated with *E. coli* O157:H7, it is necessary to provide the food industry with procedures for preventing its presence and controlling its growth. The growth kinetics of the serovar O157:H7 under variable environmental conditions (incubation temperature, initial pH, NaCl content) have been investigated. This has allowed the subsequent development of mathematical models that predict its behaviour in foods (Buchanan and Klawitter 1992; Buchanan *et al*. 1993; Sutherland *et al*. 1995).

Knowing the $T_{\min}$ (the temperature below which growth is no longer observed) and $T_{\max}$ (the temperature above which no growth occurs) for *E. coli* O157:H7 is particularly important in order to preserve food and to detect this bacterium. T_{opt} (the temperature at which the maximum specific growth rate μ_{max} equals its optimal value) is an important parameter in predictive microbiology. A strong correlation has been observed between the three cardinal temperatures ($T_{\min}$, $T_{\max}$ and T_{opt}) on several strains of various bacterial species (Rosso *et al*. 1993). Their values for three non-O157 *E. coli* strains were evaluated between 4·9°C and 11·2°C for $T_{\min}$, 47·3°C and 48·0°C for $T_{\max}$ and 40·3°C and 41·3°C for T_{opt} (Rosso *et al*. 1993). The inability of *E. coli* O157:H7 to grow well, if at all, at 44–45·5°C was reported for trypticase soy broth (TSB) (Doyle and Schoeni 1984) and for E coli medium (Raghubeer and Matches 1990). The results, obtained in each case with a single strain were later interpreted to mean that the cardinal temperatures for this serovar would be lower. In contrast to these findings, Palumbo *et al*. (1995) observed that most O157 strains grew over a range of 8 to 10°C to at least 45°C in brain heart infusion (BHI). Moreover, growth of O157:H7 isolates was adequately described by a model for non-pathogenic *E. coli* strains (Salter *et al*. 1998), and at the same time, growth of non-pathogenic strains was adequately described by a model for pathogenic *E. coli* strains (Buchanan *et al*. 1993; Sutherland *et al*. 1995). Such divergent data could be explained through variability between strains and culture conditions. In fact, Ferenc *et al*. (2000) observed that the ability of *E. coli* O157:H7 to grow at 45·5°C depended on the medium, the inoculum size and the strain. At this temperature, only three of the 18 strains studied grew well in EC broth, with an initial density of 100 cfu ml^{-1}, while all strains grew with higher initial densities. In TSB, all the strains were able to grow even with an initial density of 10 cfu ml^{-1}.

This study was undertaken to compare the T_{opt} of serovar O157 with other *E. coli* serotypes in a complex laboratory

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medium. In fact, the precision of T_{opt} experimental values were better than those of T_{min} and T_{max}. Several strains were included to account for the described variability between isolates (Palumbo *et al.* 1995; Salter *et al.* 1998).

MATERIALS AND METHODS

Bacterial strains

Thirty-two *Escherichia coli* strains of different origins were studied, 20 belonging to serotype O157 (19 O157:H7 and one O157:H−) and 12 belonging to other serotypes (Table 1). The strains were checked for the presence of the O157 antigen through latex agglutination using the *E. coli* latex kit (Oxoid), and for the presence of the H7 antigen through direct immunofluorescence. PCR confirmed that all the O157 strains contained the *eaeA* gene (Deng and Fratamico 1996), a modified *uidA* gene (Cebula *et al.* 1995) and the *fliC* gene (Fields *et al.* 1997). Results for the Shiga-like toxin genes (*stx1* and *stx2*), also determined by PCR (Cebula *et al.* 1995), are shown in Table 1. Bovine as well as human isolates were included in the two groups (Table 1). All the strains were epidemiologically unrelated. They were stored at −80°C in 10% (v/v) glycerol brain heart infusion (bioMérieux).

Growth medium and growth conditions

All the experiments were conducted in the same batch of Mueller-Hinton broth (Biorad). Cultures were grown in 250 ml Erlenmeyer flasks. They were incubated in a water bath (Polystat μpros, Bioblock Scientific, Illkirch, France) regulated at ± 0·1°C, and homogenized by magnetic stirring at 300 rev min^{-1}.

Spinal needles (Becton Dickinson, San Jose, CA, USA) were used to inoculate and retrieve samples without removing the flask from the incubator. Each needle was used once in order to avoid contamination.

Flasks were inoculated with 1 ml of *E. coli* suspension in buffered peptone water (Merck) to yield an initial count of approximately 2×10^6 cfu ml^{-1}. The cells were harvested from a stationary phase culture obtained from the stock suspension grown overnight on blood agar plates (bio-Mérieux) at 37°C.

For each strain, the maximal specific growth rate (μ_{max}) was determined at 8–12 temperatures between 33 and 47°C, with an increment of 1°C each time.

Estimation of the optimal growth temperature

Growth was monitored by periodic measurement of O.D. at 450 nm.

For each strain and each temperature, a semi-log curve of O.D. *vs* time was plotted. The maximal specific growth rate value (μ_{max}) was first calculated by linear regression analysis; for each growth curve, more than 10 O.D. measurements between 0·05 and 0·3 were taken into account.

For each bacterial strain, the optimal growth temperature was then calculated using the CTMI model to describe the growth rate values obtained at different temperatures (Rosso *et al.* 1993):

$$\mu_{max}(T) = \mu_{opt} \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]}$$

where $\mu_{max}(T)$ (h^{-1}) is the maximum specific growth rate at temperature T (°C), T_{min} (°C), T_{max} (°C) and T_{opt} (°C) are the cardinal temperatures, and μ_{opt} (h^{-1}) the value of the maximum specific growth rate μ_{max} reached at T_{opt}. As the growth curves were done only around T_{opt}, the CTMI model was re-parameterized by taking into account the high linear correlation which had previously been observed between the three cardinal temperatures (Rosso *et al.* 1993). In the mathematical formulation of the model, T_{min} was replaced by 0·953 T_{opt} − 28·9 and T_{max} by 1·101 T_{opt} + 3·2. A simplified form of the CTMI model with only two parameters, μ_{opt} and T_{opt}, was thus fitted for each bacterial strain by non-linear regression. Minimizing the sum of the squared residuals was computed by calls to the NAG Foundation Library routine E04FDF (Numerical Algorithms Group Ltd, The Math Works Inc., Natick, MA, USA). Confidence intervals for parameter estimations were computed using the variance-covariance matrix given by the NAG Foundation Library routine E04YCF.

The reproducibility of T_{opt} measurement was determined by three complete replicate trials for two strains (strains 17 and 29).

In order to compare the T_{opt} mean values obtained for O157 and non-0157 strains, the robust Mann–Whitney *U*-test was used.

RESULTS

For all strains, the growth curve obtained at each temperature was log linear. The coefficient of regression was always above 0·99, which allows the maximum specific growth rate to be estimated precisely. As an illustration, the maximum specific growth rates obtained for two strains (the O157 strain 17 and the non-O157 strain 29) at different temperatures are reported in Fig. 1, with the confidence interval for each growth rate value.

T_{opt} values were determined with relative precision, as can be seen from the confidence intervals plotted on Fig. 2. During the reproducibility assay, the differences between the two extreme T_{opt} values were 0·4°C (strain 29) and 1·1°C (strain 17). For the 32 strains studied, the T_{opt} values were

Table 1 Origins and characteristics of the 32 tested strains

Group	Strain*	Origin	Serotype	*Stx*	
				stx1	*stx2*
O157	1	Reference strain (ATCC 43895)	O157:H7	+	+
	2	Bovine faeces	O157:H7	–	–
	3	Bovine faeces	O157:H7	–	–
	4	Bovine faeces	O157:H7	–	+
	5	Bovine faeces	O157:H7	–	+
	6	Bovine faeces	O157:H7	–	+
	7	Bovine faeces	O157:H7	–	+
	8	Bovine faeces	O157:H7	–	+
	9	Bovine faeces	O157:H7	–	+
	10	Bovine faeces	O157:H7	–	+
	11	Bovine faeces	O157:H7	–	+
	12	Bovine faeces	O157:H7	–	+
	13	Ground beef	O157:H7	–	+
	14	Ground beef	O157:H7	–	+
	15	Bovine slaughterhouses	O157:H7	–	+
	16	Bovine slaughterhouses	O157:H7	–	+
	17	Bovine slaughterhouses	O157:H7	–	–
	18	Human diarrhoea	O157:H7	–	+
	19	Human diarrhoea	O157:H7	+	+
	20	Human diarrhoea	O157:H-	–	+
Non-O157	21	Human diarrhoea	O103:H2	+	–
	22	Human diarrhoea	O26:H11	+	–
	23	Human diarrhoea	O111	+	+
	24	Calf septicaemia	nd	–	–
	25	Calf septicaemia	nd	–	–
	26	Calf septicaemia	nd	–	–
	27	Bovine mastitis	nd	–	–
	28	Bovine mastitis	nd	–	–
	29	Bovine mastitis	nd	–	–
	30	Chamois	nd	+	–
	31	Calf faeces	K99	–	–
	32	Reference strain (NC 4100)	K12	–	–

*Strains 18–20 were kindly supplied by J. Freney (Hôpital Edouard Herriot, Lyon, France), the strains 21–23 by M. Lange (Institut Pasteur, Lille, France) and the strains 24–29 and 31 by J.L. Martel (Agence Française de Sécurité Sanitaire des Aliments, Lyon, France).
nd: not determined.

found to be between 38·5°C (strain 14) and 41·5°C (strain 19), with a mean of 40·2°C for the O157 group and between 40·4 (strain 25) and 41·9°C (strain 24) with a mean of 41·2°C for the non-O157 group. These two mean values are significantly different ($P = 0{\cdot}0002$).

For the optimal growth rate (μ_{opt}), the values obtained were between 1·5 and 2·0 h^{-1} for the O157 and between 1·2 and 2·1 h^{-1} for non-O157 strains, with a mean of 1·8 h^{-1} for both serogroups. These growth rates correspond to generation times (GT) between 19·8 and 34·7 min, with an identical mean of 23·1 min.

DISCUSSION

As these findings indicate, the O157 and non-O157 *E. coli* differed in optimal growth temperatures but not in optimal growth rates. The observations of Doyle and Schoeni (1984) and Raghubeer and Matches (1990) with T_{opt} values around 37°C for O157 strains were not confirmed. Our results corroborate previous reports (Palumbo *et al.* 1995; Salter *et al.* 1998). The existence of strains with extreme T_{opt} values (such as strain 14) observed in our study in the O157 serogroup could explain the observations of Doyle and

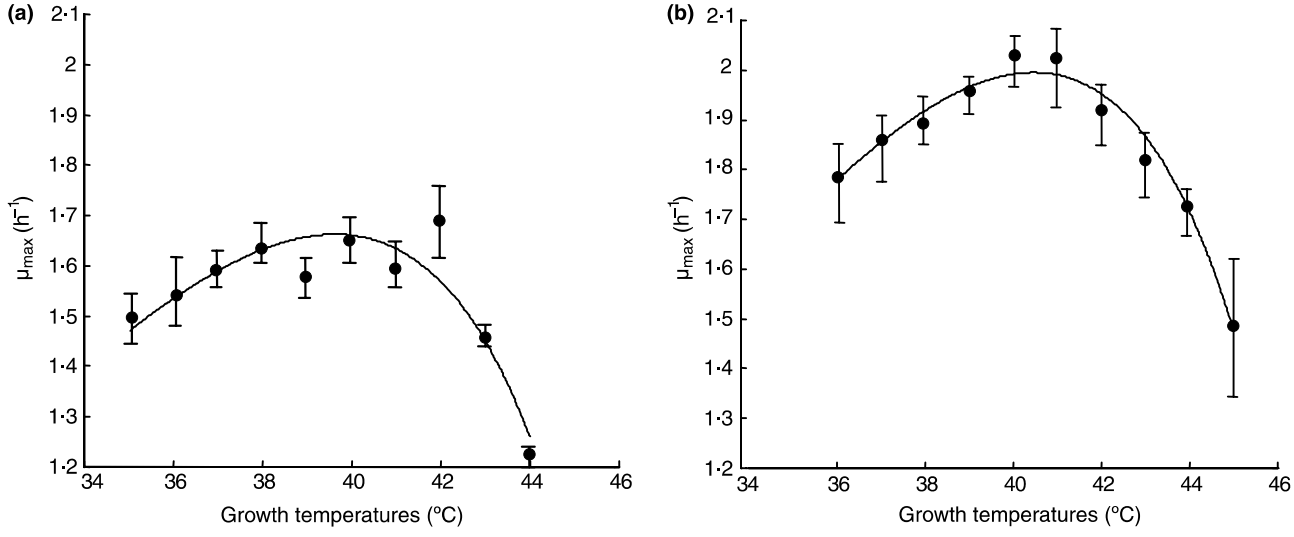

Fig. 1 Maximum specific growth rates (μ_{max}) observed for (a) strain 17 and (b) strain 29 at different temperatures with the CTMI model fitted to these values. Bars correspond to the confidence intervals

Schoeni (1984) and Raghubeer and Matches (1990) made on only one strain of *E coli* O157:H7. The T_{opt} variability between strains seems to be more important in the O157 group, with a standard deviation of 0·654 for O157 and of 0·513 for non-O157 strains. Nevertheless, this difference is not significant ($P = 0{\cdot}38$ with the Fisher test) and the trend should be confirmed.

The significant difference of T_{opt} values for O157 and non-O157 *E. coli* observed in this study could not be related to the origin of the strains as the two groups included bovine as well as human isolates. In fact, the T_{opt} difference between O157 and non-O157 strains from bovine origin only (strains 1–17, and strains 24–29 and 31) was also statistically significant ($P = 0{\cdot}003$), with a mean of 40·1°C for O157 and 41·1°C for non-O157 strains.

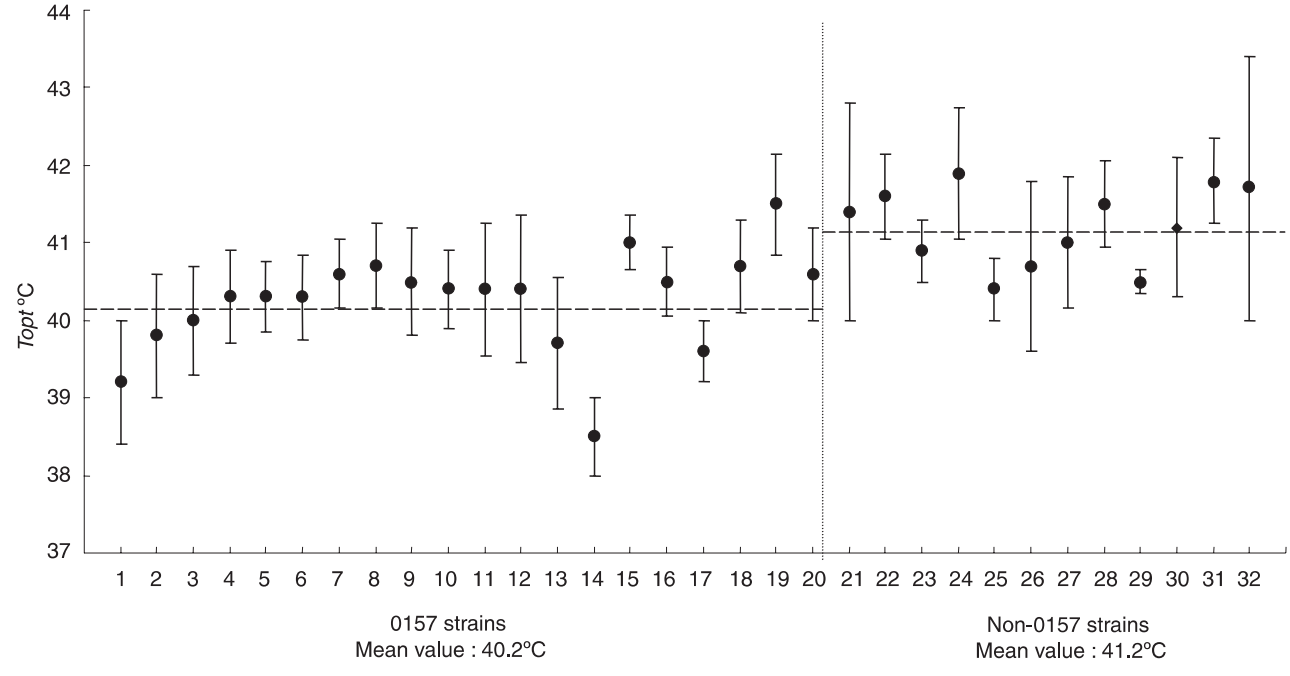

Fig. 2 Optimal temperatures (T_{opt}) determined for each strain. Bars correspond to the confidence intervals. The dotted lines correspond to the mean values for each group

The small difference in T_{opt} values for O157 and non-O157 *E. coli* appeared to have no biological implications, nor could it be applied to the field of predictive microbiology. In particular, the results do not allow the classical methods used for isolating faecal coliforms to be asserted as suitable for the detection of *E. coli* O157:H7.

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