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Demonstration of *cel* Operon Expression of *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* at Elevated Temperatures Refractory to Their Growth

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When *Escherichia coli* was incubated at the growth-refractory temperatures of 48 and 54°C, expression of the *cel* operon was demonstrated by phospho-β-glucosidase activity. This enzyme activity was also detected at the growth-refractory temperatures in *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Thermotolerant and mesothermophilic mutants of *E. coli*, *S. typhimurium*, and *P. aeruginosa*, able to grow with generation times of 30 to 40 min at 48 and 54°C, exhibited phospho-β-glucosidase activity at their growth temperatures of 48 and 54°C. Thus, the *cel* operon previously described as a cryptic operon in *E. coli* and *S. typhimurium* was found to be expressed at growth-refractory temperatures of the mesophilic parent and growth-permissive temperatures (48 and 54°C) of the thermotolerant and mesothermophilic mutants.

Hall and coworkers have analyzed the *cel* operon in *Escherichia coli* and demonstrated the cryptic nature of the wild-type *cel* operon mRNA synthesis at mesophilic temperatures (10). When mesophilic *Escherichia coli* strains were grown at 37°C on 1% cellobiose-supplemented MacConkey agar, the colonies were uncolored, implying that cellobiose was not catabolized. These colonies turned bright pink when shifted to 48°C for a 3- to 4-h incubation. However, if 200 μg of rifampin per ml was sprayed on these colonies and the colonies were then incubated at 48°C for 3 to 4 h, the color of the colonies remained unchanged. Thus, it appeared that mRNA synthesis was a prerequisite for expression of the *cel* operon at elevated temperatures.

These observations implied that, though these elevated temperatures were refractory to growth of mesophilic *E. coli*, the *cel* operon was expressed. Thus, we assayed for phospho-β-glucosidase activity by the use of an in vivo hydrolysis assay of *p*-nitrophenyl-β-glucopyranoside as described by Parker and Hall (11). The cells were grown to late log phase (10^9 cells per ml) in nutrient broth at 37°C, centrifuged, washed, and resuspended in minimal salts medium (11) for assay of phospho-β-glucosidase; the suspension was incubated for 1 h at 37, 48, or 54°C and centrifuged to remove the cells, and the A_{600} was read. As can be seen in Table 1, mesophilic *E. coli* showed considerable phospho-β-glucosidase activity at both 48 and 54°C but not at 37°C. The amount of activity in the mesophiles increased as the temperature of the assay increased from 48 to 54°C. Thus, mesophilic *E. coli* expressed the *cel* operon at elevated temperatures refractory to its growth. Hall and Betts (10) have demonstrated that mRNA for the *cel* operon is not synthesized at mesophilic temperatures in the wild-type *E. coli*. We observed no phospho-β-glucosidase activity when 20 μg of rifampin per ml was added prior to temperature elevation (Table 1). This information suggests that expression of *cel* operon is regulated by temperature at the transcriptional level.

Schaefler and Malamy (13) have described the presence of a cryptic *cel* operon in another member of the family *Enterobacteriaceae*, *Salmonella typhimurium*. We found catabolism of cellobiose in *S. typhimurium* and also *Pseudomonas aeruginosa* when colonies were incubated on MacConkey agar with 1% cellobiose at the refractory temperature of 48°C. At 37°C no catabolism of cellobiose was observed in these species. Furthermore, Table 1 shows that *S. typhimurium* and *P. aeruginosa* mesophiles exhibited phospho-β-glucosidase activity when incubated at the growth-refractory temperatures (48 and 54°C) but not at the mesophilic temperature (37°C).

From mesophilic *E. coli* strains, we have isolated thermotolerant and mesothermophilic mutants which are capable of growth (generation times, 30 to 40 min) at 48 and 54°C, respectively (5–7). Similar thermotolerant and mesothermophilic mutants of *S. typhimurium* and *P. aeruginosa* have been isolated (5, 6). In these thermal environments, none of these mutants were able to grow on minimal medium with glucose or glycerol (unpublished observation). This observation was in agreement with our previous finding that thermophilic *Bacillus subtilis* and *Bacillus pumilus* mutants capable of growth on rich medium between 50 and 70°C would not grow on minimal medium plus glucose above 55°C (4).

Catabolism of cellobiose under thermal conditions by the mesophilic *Escherichia*, *Salmonella*, and *Pseudomonas* strains strongly indicated that cellobiose is utilized in the thermal environments. When cellobiose was added as the sole carbon source, the thermolerant and mesothermophilic strains all formed good-sized colonies in about 24 h on minimal medium (7 g of K_2HPO_4, 1 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, 1 g of $MgSO_4$, 1.5% granular agar; Difco) at 48 and 54°C (5, 6). The genetic origin of these thermotolerant and mesothermophilic strains was also confirmed by demonstration of appropriate auxotrophic requirements in the thermal environments on minimal medium containing 1% cellobiose (5, 6). Furthermore, as shown in Table 1, the thermotolerant and mesothermophilic mutants of all these genera exhibited phospho-β-glucosidase activity at 48 or 54°C but not at 37°C. Thus, it appears that expression of the

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TABLE 1. Induction of phospho-β-glucosidase activity in mesophilic, thermotolerant, and thermophilic strains

Strain	Sp act[a] after incubation for 1 h at temp (°C)		
	37	48	54
E. coli			
B[b]	0.029 ± 0.019	5.55 ± 0.48	7.4 ± 0.91
B T/48	0.094 ± 0.049	5.2 ± 0.76	ND
B T/54	0.129 ± 0.97	ND	4.1 ± 0.78
S. typhimurium			
Q	0.037 ± 0.032	1.85 ± 0.11	3.4 ± 0.36
Q T/48	0.251 ± 0.22	2.54 ± 0.37	ND
Q T/54	0.172 ± 0.15	ND	7.5 ± 0.46
P. aeruginosa			
POA	0.046 ± 0.032	2.5 ± 0.45	6.3 ± .89
POA T/48	0.154 ± 0.099	3.6 ± 0.91	ND
POA T/54	0.143 ± 0.079	ND	4.7 ± 0.89

[a] Units of phospho-β-glucosidase activity/A_{600} ± 95% confidence limits. Duplicate assays were performed. The enzyme substrate for the assay (*p*-nitrophenyl-β-glucopyranoside) was thermostable under these assay conditions. ND, not determined.

[b] Addition of 20 μg of rifampin per ml to the cells prevented *cel* operon expression in the mesophiles at the elevated temperatures.

cel operon in thermal environments is of consequence to growth in the thermal environments.

It has been previously shown that an altered DNA gyrase activity resulting from a mutation of the *gyrA* gene can result in thermotolerant mutant strains (5, 7). However, since enzymatic activity of phospho-β-glucosidase was readily detected in mesophilic strains by incubation at the growth-refractory temperatures, expression of the phospho-β-glucosidase gene is apparently a direct response to increased temperature, independent of the mutation to thermotolerant growth. DNA superhelicity has been shown to regulate gene expression (1, 8). The expression of genes is also known to be regulated by the growth temperature (2, 9). Increased temperature can itself alter DNA superhelicity (3). It has been demonstrated that the cryptic *bgl* operon of *E. coli* can be decryptified by alterations in DNA superhelicity (12). Thus, it is inferred that the phospho-β-glucosidase activity observed in mesophilic strains resulted directly from temperature-altered DNA superhelicity and the *cel* operon can be decryptified by incubation of these mesophiles at growth-refractory temperatures.

In this communication, we have presented data showing that the *cel* operon of *E. coli*, previously described as cryptic when studied at mesophilic temperatures (10), is expressed or decryptified in thermal environments where mesophilic *E. coli* is unable to grow. Since we found that the genetically well-established genera *Escherichia*, *Salmonella*, and *Pseudomonas* are all capable of mutating to grow in thermal environments up to at least 54°C (5–7), availability of these genetically well-characterized systems provides us a tool to study the nature of evolutionary conserved genes.

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