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Transcriptional regulation of the pO157-encoded ecf1 fragment by intrinsically curved DNA in Escherichia coli O157:H7

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Introduction

It remains to be elucidated how *Escherichia coli* O157:H7 thrives in diverse temperature environments such as the human and ruminant gastrointestinal tracts, in food, and in the farm environment. Recently, thermoregulation of virulence-associated genes by intrinsic DNA curvature was demonstrated in some enteric pathogens. We previously demonstrated that the 60-megadalton plasmid (pO157) in *E. coli* O157:H7 contains multiple intrinsically curved DNAs and identified them using a standard linker primer PCR technique. Among the identified DNA fragments, one was designated as bent 2 fragment (bnt-2) and further characterized.

Materials and Methods

Two dimensional gel electrophoresis suggested that a 532-bp region at the 3′-end of BNT2, which contains several in-phased homopolymeric dA:dT-tracts, was primarily responsible for the retarded electrophoretic mobility at 4℃. Since this curved DNA region contains a putative promoter, we analyzed its promoter function using an operon fusion system and also examined transcriptional responses to different growth conditions.

Results and Discussion

The results showed that the BNT2 fragment has a functional promoter that showed increased activity at

24℃compared to 37℃. A homology search of the BNT2-driven genes, previously designated as eae-positive conserved fragments (*ecf*), suggested that they may be associated with exopolysaccharide or lipopolysaccharide (LPS) synthesis. Thus, the LPS profiles and the sensitivity to deoxycholate (DOC) in wild type *E. coli* O157:H7 and its pO157-cured strain were compared at 37℃ and 24℃. The results showed that there was no difference in the LPS profiles, but when grown on 0.5% DOC-containing LB plates, the pO157-cured strain produced characteristic slimy colonies and grew better than the wild type strain at 24℃. Further studies will be required to explain the relationship between this phenotype and the ecf fragments.

REFERENCES

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