SL332

The Structure and Regulation of Lysine Decarboxylase Gene in *Salmonella typhimurium*

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Salmonella typhimurium responds to changes in external pH by altering its pattern of gene expression. To better understand how bacteria sense and respond to changes in external pH, we have initiated studies involving direct screening for acid-induced genes using the technique of Mu*d*-*lac* operon fusion analysis and we have been studied the genetic elements involved in acid induction of the *S. typhimurium cad* operon encoding lysine decarboxylase.

Five acid-induced genes(*aci*) in *S. typhimurium* have been identified. They include *aci*A(99min), *aci*B(90~93min), *aci*J, *aci*K(33~36min), *cad*A(54min). All were induced by low pH condition. These genes exhibited strict co-inducer requirement for small molecules to be expressed in minimal medium. These included tyrosine for *aci*K, lysine for *cad*A, and unknown components of complex medium for *aci*A and *aci*B.

The *cad*A locus was subsequently shown to encode LDC as evidenced by the loss of LDC activity in the *cad* insertion mutants. *S. typhimurium cad*BA was cloned by colony hybridization, using the probe of *cad*BA gene of *E. coli*. The 4.3kb *Bam*HI-*Sal*I fragment from pPF86 was subcloned into pBR322. We report the sequence of the *cad* operon encoding lysine decarboxylase, a protein of 714 amino acids, and another protein, *cad*B, of 445 amino acids. The amino acid sequence of lysine decarboxylase showed high homology to that of lysine decarboxylase of *E. coli*, and *Hafnia alvei*. The CadB sequence showed homology to that of CadB of *E. coli*, and ArcD of *Peudomonas aeroginosa*, encoding lysine/cadaverine antiporter and arginine/ornithine antiporter, respectively.

The expression of *S. typhimurium cad*BA operon requires at least two extracellular signals : low pH and high concentration of lysine. To further characterize the nature of pH-dependent and lysine-dependent signal transduction, the mutants which express a *cad*A-*lac*Z operon fusion in various growth conditions by Tn*10* insertion, spontaneous mutagenesis, and EMS treatment were isolated. The *cad*C::Tn*10* mutants were not inducible by low pH and this insertion were complemented by a cloned *cad*C locus from *E. coli*. Thus CadC is positive regulator of *cad*A expression.

Two *cad*R::Tn*10*, *cad*R3 and *cad*R4 mutants were isolated from which *cad*A-*lac*Z was expressed in the absence of exogenously added lysine. They were also resistant to thiosine and complemented by *lys*P clone from *E. coli*. Thus, in the absence of exogenous lysine, *cad*R is a negative regulator of *cad*BA expression. *cad*R::Tn*10* were unlinked to the *cad*A-*lac*Z insertion and was mapped to between 55 and 57min. using Mu*d* PQ System. The other mutation(*cad*C3 and *cad*C4) in *cad*C conferred pH-independent and lysine-dependent *cad*BA expression. The *cad*BA expression was affected by osmolarity and induced by low osmolarity. The *cad*A locus was very sensitive to oxygen levels and was cleary anaerobically induced. Cadaverine, the product of lysine decarboxylation, was shown to inhibit expression of *cad*A-*lac*Z fusion in *cad*C^{+} cell.