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Note

Chromosomal Mapping of the *cdd* Gene Encoding Deoxycytidine-Cytidine Deaminase in *Bacillus subtilis*

Song Bang- Ho[1*] and Jan Neuhard[2]

[1]Department of Biology, Teachers College, Kyung-pook University, Taegu 702-701, Korea
[2]Enzyme Division, Institute of Biological Chemistry B,
University of Copenhagen, Copenhagen, Denmark

*Bacillus subtilis*의 시티딘 디아미나제를 코드하는 *cdd* 유전자의 Chromosomal Mapping

송방호[1*]·얀 노이하드[2]

[1]경북대학교 사범대학 생물교육과 [2]덴마크 코펜하겐대학 생물화학연구소B, 효소분과

A mutant of *Bacillus subtilis* with a defective *cdd* gene encoding deoxycytidine-cytidine deaminase (EC 3.5.4.5) has been characterized genetically. The genetic lesion, *cdd*, causing the altered deoxycytidine-cytidine deaminase was mapped at 225 min on the linkage map of *B. subtilis* by AR9 transduction. Transductional analysis of the *cdd* region established the gene order in clockwise as *trp-lys-cdd-aroD*. The *cdd* gene was linked 72% with the *aroD* and 20% with the *lys*.

In this study the *cdd* gene of *Bacillus subtilis*, encoding deoxycytidine-cytidine deaminase ((deoxy) cytidine aminohydrolase (EC 3.5.4.5)), was mapped in its chromosomal location. The enzyme converts cytosine nucleotides into the corresponding uracil nucleotides (1-2).

The *cdd* gene of *B. subtilis* was cloned and characterized recently by Song and Neuhard(3a). However, the gene locus was completely unknown yet. The *cdd* gene was inserted into the EcoRI/AvaI sites of pBR322 and the nucleotides coding for monomer polypeptide were sequenced. The theoretical molecular mass of *cdd*, deduced from the nucleotide sequence of monomer, was 14,837. The value corresponded to the calculated molecular mass of 14 kDa in the mini cell experiment. By comparing it to the molecular mass of the native form in sucrose density gradient or gel filteration, the *cdd* gene product may be composed of four identical polypeptides as a tetramer(3b). In this paper, AR-9 transduction was used to determine the *cdd* gene location in the chromosomal map by detecting the cotransducing rate with *trp, lys,* and *aroD*.

The cytidine deaminase of *Escherichia coli*, originally detected by Wang and Lampen(4), has been purified and characterized intensively(5,6). A homogeneous preparation of the enzyme was calculated to have the molecular mass of 35,000 as a monomer determined in sodium dodesyl sulfate poly acrylamide gel electrophoresis and 54,000 as a native form in gel filteration(7). The result was very similar to other reports(8). This suggested that two apparently identical subunits with molecular mass of 35,000 could be combined each other and recognized as a dimer in the native form of the *cdd* gene product. The promoter region of *cdd* structural gene was cloned to the polylinker cloning site of *lacZ* fusion vector for a study of gene regulation(9), because the *cdd* gene is involved in the catabolism of nucleosides and regulated by the repressor encoded by the *cytR* gene(10,11), or by the cyclic AMP-binding protein(12). The *cdd* gene in *E. coli* K-12 is located counterclockwise to *ptsF* between 46 min and 47 min. The gene order in the region of the *E. coli* chromosome was found to be *his-get-dld-*

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* Corresponding author

cdd-ptsF(13).

The bacterial strains used were all derivatives of *Bacillus subtilis*168 (Table 1). The transducing host used was in most cases *B. subtilis* ED40 which is a *pyr cdd* double mutant required either uracil or uridine for growth as a sole pyrimidine source. For proliferation of AR9 phage, *Bacillus pumilus* (ED153) was used as the host. The bacteria were grown in the Spizizen minimal medium supplemented with glycerol as a sole carbon source and with the requirements for the strains being tested. Selection and testing of the genotypes were based on the following phenotypic traits; cdd^+ strains can utilize 0.1% cytidine as a sole pyrimidine source; *lys, trp, ilv,* and *leu* strains can grow on the medium contain corresponding amino acids as requirements, *aro*D requires tyrosine, phenylalanine, and tryptophane. Recombinants from transductions were purified at least once before use.

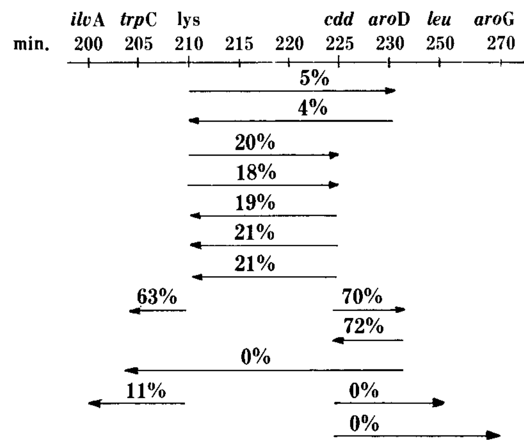

Fig. 1. The *cdd* gene location on the chromosomal map of *Bacillus subtilis*.
Values are percentages of joint transduction based on data from Table 2. Arrow heads point to the unselected markers.

Recipients were grown to the stationary phase overnight in Luria broth supplemented with glycerol as a sole carbon source, and then the cells were inoculated into brain heart infusion broth (BHIB) supplemented with 0.4% yeast extract. An aliquiot of AR9 phage stock was transferred to the culture. The mixed culture was shaken for 8 to 10 hrs at 30°C, and then incubated without shaking at 30°C overnight. A few drops of chloroform were added and cell debries was removed. After re-chloroform treatment the cleared supernatant was used as a transducing lysate.

General transduction in *B. subtilis* with phage AR9 was done essentially as described by Saxild and Nygaard(14). Recipient cells were grown overnight at 30°C in MGl medium(15) supplemented

Table 1. Strains of *Bacillus* spp. used

	Strain	Genotype	Source
Bacillus subtilis	ED 39	*pyr*2, *lys*	T. Takahashi
	ED 40	*pyr*2, *cdd*1, *lys*	B. Rima *et al*
	ED 69	*pur*A16, *cys*A14, *trp*C2	F. Kunst
	ED 70	*pyr*2, *cdd*1, *lys, dcd*1, *nrd*3	F. Kunst
	ED 71	*aro*I906, *pur*B33, *dal, trp*C2	F. kunst
	ED 72	*tre*12, *met*C3, *gly*B133, *trp*C2	F. Kunst
	ED 73	*pyr*D, *ilv*A1, *thy*A, *thy*B, *trp*C2	F. Kunst
	ED 74	*glt*A292, *trp*C2	F. Kunst
	ED 75	*aro*D120, *lys*1, *trp*C2	F. Kunst
	ED 76	*leu*A8, *aro*G932, *ald, trp*C2	F. Kunst
	ED 77	*his*A1, *thr*5, *trp*C2	F. Kunst
	ED 78	*sac*A321, *ctr*A1, *trp*C2	F. Kunst
	ED 133	*pyr*2, *aro*D120, *lys*1	own collection
	ED 192	*ilv*A1, *sac*A78, *gua*A1	own collection
	ED 211	*pyr*2, *cdd*1	this work
	ED 212	*dna*E20, *ilv*A1, *met*B5	BGSC[a]
Bacillus pumilus	ED 153	host of bacteriophage AR9	own collection

[a] Obtained from the *Bacillus* Genetic Stock Center

with their requirements. A 0.2 m*l* volume of the above phage lysate and 0.5 m*l* of these cell cultures were mixed and incubated for 30 min at 30C. The cells were spun down and washed with Spizizen minimal medium(16), and then plated out on the selective plates, after resuspending the cells with the minimal medium.

The *cdd* gene location on the chromosome of *B. subtilis* was determined by transducing it with nine reference strains of Dedonder *et al.*(17) as donors and with strain ED40 as a recipient. A *B. subtilis cdd* mutant lacking both cytidine and deoxycytidine deaminase activity has previously been isolated and characterized by Rima and Takahashi (18). Phenotyphically the *cdd* mutant was unable to utilize cytidine or deoxycytidine as sole pyrimidine source. Thus a *pyr cdd* double mutant requires either uracil or uridine for growth; cytidine or deoxy cytidine will not satisfy the pyrimidine requirement. In the first recombination, each locus in the kit strains transduced to the ED40, and then selected cdd^+ cells on the plate supplemented with cytidine. In phage AR-9 mediated transductions, it was found that the *cdd* mutation in the recipient cotransduced with *lys* in high frequency.

In the second cross, the cotransducing frequency of *cdd* with *lys* was revealed 21% (from ED76 to ED40), 19% (from ED192 to ED40), and 18% (from ED77 to ED40). The *lys* mutation cotransduced with both *cdd* and *ilv*A (11% from ED192 to ED40), whereas no cotransduction betweeen *ilv*A and *cdd* was observed (Table 2). In the reverse selection the *lys* was linked 20% with *cdd* in transduction from ED192 to ED40 and 18% from ED76 to ED40. Consequently, *cdd* gene was linked with *lys* about 20% and the same transducing ratio was revealed in reverse cross. However, the *cdd* was not lined with *ilv*A, *leu* or *aro*G loci. Among 200 of

Table 2. Transduction of *cdd* gene by AR9 in *Bacillus subtilis*

Doner	Recipient	Selected Marker[a]	Unselected Marker						
			ilv	*trp*	*lys*	*cdd*	*aro*D	*lu*	*aro*G
ED 192	ED 40	*cdd* (89)			17/89[b] (19)[c]				
*ilv*A	*pyr, lys, cdd*	*lys* (105)	11/100 (11)			20/100 (20)			
ED 76	ED 75	*lys* (200)					10/200 (5)		
*trp*C2, *leu*A, *aro*G	*aro*D, *lys, trp*C	*aro*D (300)		0/200 (0)	8/400 (4)				
ED 76	ED 40	*cdd* (125)						0/100 (0)	0/100 (0)
*trp*C2, *leu*A, *aro*G	*pyr, lys, cdd*	*cdd* (56)		12/56 (21)					
		lys (300)		124/200 (63)		36/200 (18)			
ED 77 *his, thr, trp*	ED 40 *pyr, lys, cdd*	*cdd* (72)			14/72 (18)				
ED 40	ED 133	*cdd*-(200)					141/200 (70)		
pyr, lys, cdd	*pyr, lys, aro*D	*aro*D (300)				144/200 (72)			

[a] Numbers in parentheses give numbers of transductants tested.
[b] The ratio of transductants carring tested markers (inherited from donors) to the total numbers of selectants.
[c] Indicate cotransductional frequencies (percentage).

cdd positive colonies selected from the transduction from ED40 to ED133, 141 colonies carried *aro*D locus together, which meant that the *cdd* was linked 70% with *aro*D. In reverse test, the *aro*D was also linked 72% with *cdd*. The *lys* gene was linked 63% with *trp* (from ED76 to ED40) as shown in Fig. 1.

These results suggested that *cdd* was located around 225 degrees on the *B. subtilis* linkage map(19) in between *lys* and *aro*D loci. Our data indicate the following gene order of this region; *ilv*A-*trp*C-*lys*-*cdd*-*aro*D-*leu*-*aro*G in clockwise as shown in Fig. 1. Thus, according to the linkage map the *cdd* gene is located close to the *dna*E and *rpo*D genes. However, the precise linkage of *cdd* to these genes was not established yet.

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