JOURNAL OF BACTERIOLOGY, Apr. 1986, p. 346–348
0021-9193/86/040346-03$02.00/0

Vol. 166, No. 1

Influence of *gyrA* Mutation on Expression of *Erwinia chrysanthemi clb* Genes Cloned in *Escherichia coli*

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Received 27 June 1985/Accepted 11 December 1985

***Erwinia chrysanthemi clb* genes cloned into Nal^s *Escherichia coli* allowed growth on cellobiose, arbutin, or salicin. In contrast, Nal^r isogenic strains grew only on cellobiose. It is proposed that expression of cloned *E. chrysanthemi clb* genes is reduced by the *E. coli* chromosomal *gyrA* (Nal^r) mutation, resulting in apparent segregation of the Clb and Arb Sal characters.**

Most *Erwinia chrysanthemi* strains are able to use any one of the natural β-glucosides cellobiose (Clb), arbutin (Arb), and salicin (Sal) as a carbon source. In contrast, *Escherichia coli* does not utilize cellobiose, arbutin, or salicin, although activation of the cryptic operon *bgl* allows the cells to use both arbutin and salicin but not cellobiose (9). *E. coli*, therefore, was used as a host for in vivo cloning of *E. chrysanthemi clb* genes. An R(pULB113)-prime plasmid called pBEC2, which is able to confer a Clb$^+$ phenotype to *E. coli* K-12 and carries a 10-kilobase insert of *E. chrysanthemi* genomic DNA, was isolated (1). Genetic studies showed that, in addition to growth on cellobiose, *E. coli* strains carrying pBEC2 acquired the ability to utilize both arbutin and salicin. We have demonstrated that the *bgl* operon was not involved in those catabolisms (1). This finding suggested that, in *E. chrysanthemi* as in *Aerobacter aerogenes* (7), *clb* gene products display overlapping specificities for cellobiose, arbutin, and salicin, although these two latter sugars can probably be assimilated by another system(s) (1).

Unlike the other *E. coli* K-12 strains analyzed, LCB67 did not utilize arbutin or salicin when containing plasmid pBEC2. This strain is a W3110 Nal^r derivative; i.e., it contains a *gyrA* mutation. Mutations affecting *gyrA* or *gyrB* genes or inactivation of gyrase activity by chemical inhibitors can, by modifying DNA superhelicity, modulate the transcription efficiency of several catabolic operons, including the *bgl* operon (4, 11; for a review, see reference 5). Therefore, we suggested that in LCB67(pBEC2), the apparent segregation between the Clb$^+$ and Arb$^+$ Sal$^+$ characters could be related to the *gyrA* mutation. The purpose of this work was to ascertain the validity of this hypothesis and to offer an explanation for the observed effect.

The strains and plasmids used in this work are listed in Table 1. To allow phage P1 to develop and efficiently transduce the *gyrA* mutation, we constructed a RecA$^+$ derivative of LCB67 as previously described (3).

If the Nal^r character of LCB67 is involved in the Clb$^+$ Arb$^-$ Sal$^-$ phenotype, introduction of the same *gyrA*-mutated allele into any other *E. coli* strain carrying plasmid pBEC2 should lead to a modification of the phenotype of this strain from Clb$^+$ Arb$^+$ Sal$^+$ to Clb$^+$ Arb$^-$ Sal$^-$. Therefore, the Nal^r character was introduced by transduction into the Clb$^+$ Arb$^+$ Sal$^+$ strain LCB568(pBEC2). Forty transductants were selected on rich medium supplemented with nalidixic acid (25 μg/ml) and were subsequently tested for growth on cellobiose, arbutin, and salicin. Twelve were unable to use any β-glucoside. Plasmid analysis of one of these 12 LCB320(pBEC2) transconjugants revealed loss of the insert. Such insert instability in a Rec$^+$ background has previously been noticed by us and others with different R-prime plasmids (our unpublished results; A. Toussaint, personal communication). We assumed that such insert segregation had also occurred in the 11 remaining Clb$^-$ Arb$^-$ Sal$^-$ transductants. The other 28 Nal^r transductants were found to be Clb$^+$ Arb$^-$ Sal$^-$. Ten of them were used as donors for transferring the plasmids back into strain LCB320. From each of the 10 matings, 16 Tc^r Ap^r Km^r transconjugants were tested for growth on cellobiose, arbutin, and salicin. All of them were able to grow on the three β-glucosides. These results indicated that, in LCB67, the Clb$^+$ Arb$^-$ Sal$^-$ phenotype was actually due to the *gyrA* (Nal^r) mutation.

We then asked how the *gyrA* mutation and the Clb$^+$ Arb$^-$ Sal$^-$ phenotype were related. Our previous genetic studies have shown that at least two genes controlling cellobiose uptake (via a phosphotransferase system-dependent pathway [10]) and hydrolysis are present on the cloned insert and probably constitute an operon under the control of the cyclic AMP receptor protein-cyclic AMP complex (2). *gyrA* mutations are known to affect various cellular processes, including plasmid maintenance and gene expression. Therefore, at least three possibilities could explain the *gyrA* mutation effect: (i) reduction of the copy number of *clb* genes per cell; (ii) reduction of the expression of an *E. coli* gene encoding a general function involved as a part of cellobiose catabolism, e.g., any or all of the genes *cya*, *crp*, and *ptsIH* (encoding adenylate cyclase, cyclic AMP receptor protein, and general enzymes EI and HPr of the phosphotransferase system, respectively); or (iii) alteration of the expression of *clb* cloned genes. The first possibility, i.e., that a low copy number of cloned *clb* genes in strain LCB67(pBEC2) leads to the Clb$^+$ Arb$^-$ Sal$^-$ phenotype, was indirectly analyzed as follows. Since plasmid pBEC2 is an RP4 derivative, we would expect it to be present in one to three copies per cell (12), and so, the difference in pBEC2 copy number between Nal^r and Nal^s strains could not be more than three. Hence, if the Clb$^+$ Arb$^-$ Sal$^-$ phenotype is simply due to a gene

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TABLE 1. Bacterial strains, plasmids, and phage

Strain, plasmid, or phage	Characters	Origin
LCB67	*recA trp* Nal^r	J. Brevet
LCB67-A	Same as LCB67, but RecA$^+$	This work
LCB320	*thr leu rpsL*	J. Beckwith
LCB568	*pyrD recA rpsL*	E. Wollman
pBEC2	Tc^r Ap^r Km^r Clb$^+$	pULB113 derivative (laboratory collection)
pCLB2	Tc^r Clb$^+$	pBR322 derivative (laboratory collection)
P1	Wild type	Laboratory collection

dosage effect, increasing the copy number of *clb* genes in strain LCB67 by at least threefold should lead to a Clb$^+$ Arb$^+$ Sal$^+$ phenotype. To test this, we introduced plasmid pCLB2, a high-copy-number pBR322 derivative which carries the same *E. chrysanthemi clb* insert as pBEC2 (Barras et al., Proc. 6th Int. Conf. Plant Pathol. Bacteria, in press), by transformation into both strains LCB67 and LCB568. Tc^r transformants were streaked on minimal medium containing one of the three β-glucosides as a carbon source. The increase in copy number led to faster assimilation of cellobiose and arbutin by strain LCB67(pCLB2), but growth on salicin remained poor even after 48 h of incubation (Table 2). We should point out that *E. coli* K-12 possesses a constitutively expressed *bglA* gene encoding an arbutin P hydrolase (8), which probably contributes to growth of LCB67(pCLB2) on arbutin. Despite that, the most significant observation was that the growth rate on cellobiose of LCB67(pCLB2) (which probably carries more than 30 copies of the *clb* genes) was equivalent to that of LCB568(pBEC2) (which carries one to three copies of the *clb* genes) (Table 2). Thus, it is apparent that a gene dosage effect cannot account for the Clb$^+$ Arb$^-$ Sal$^-$ phenotype observed.

The second possibility, i.e., reduction of the expression of any or all of the genes *ptsIH*, *cya*, and *crp* in strain LCB67, was analyzed by testing the ability of this strain to utilize carbohydrates which are either transported by the phosphotransferase system, for instance, mannitol, or catabolized by a cyclic AMP receptor protein-cyclic AMP complex-regulated pathway, for instance, lactose. We observed that strain LCB67 grew on mannitol and lactose as well as did the other *E. coli* strains, LCB568 and LCB320. These observations allowed us to rule out the second possibility.

We then considered that, in strain LCB67, the *gyrA* mutation affected the expression of various catabolic genes, including the cloned *clb* genes. The rate of utilization of maltose by LCB67 was tested, since previous studies showed that expression of the *mal* regulon is sensitive to nalidixic acid at sublethal concentrations (11). We observed that strain LCB67 failed to produce the characteristic metallic sheen when grown on maltose-supplemented EMB medium and that this organism grew poorly on minimal medium supplemented with this carbon source. A similar result was obtained when xylose was used instead of maltose. This observation supports the third hypothesis, namely, that in this strain the *gyrA* mutation reduces the expression of certain chromosomal catabolic operons and, in the same way, affects the expression of cloned *E. chrysanthemi clb* genes.

TABLE 2. Time of incubation (at 37°C) required for *E. coli* Clb$^+$ clones to produce normal-size colonies on minimal medium supplemented with one of the β-glucosides as a carbon source

Strain	Incubation time (h) required with:		
	Cellobiose	Arbutin	Salicin
LCB67(pBEC2)	48	NG[a]	NG
LCB67(pCLB2)	24	36	NG
LCB568(pBEC2)	24	36	24
LCB568(pCLB2)	16	24	16

[a] NG, No growth.

How could reduction of expression by *clb* genes lead to a Clb$^+$ Arb$^-$ Sal$^-$ phenotype? One explanation might be that, in *E. chrysanthemi* as in *A. aerogenes* (7), *clb* gene products have an affinity for cellobiose that is much stronger than that for both arbutin and salicin. If so, a low enzyme concentration, due to a low level of expression of *clb* genes, could in turn result in less growth on cellobiose and no growth at all on the other β-glucosides.

Although we are aware that interpreting gene expression in terms of DNA superhelicity is not straightforward, it is tempting to propose that the expression of *E. chrysanthemi clb* genes cloned in *E. coli* is modulated by the level of DNA supercoiling. It would be of interest to test whether the same regulation can be observed in *E. chrysanthemi* when the *clb* genes are in their normal chromosomal location.

We thank Arun K. Chatterjee and his colleagues for critically reading the manuscript and A. Toussaint for her interest in this work.

This research was supported by research contract no. GBI-3-0015-F of the Biomolecular Engineering Programme of the Commission of the European Communities and by ATP Microbiologie of the Institut National de la Santé et de la Recherche Médicale-Centre National de la Recherche Scientifique.

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