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Cloning and Characterization of *araA*, *araB*, and *araD*, the Structural Genes for L-Arabinose Utilization in *Bacillus subtilis*

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Two recombinant plasmids, pSNL1 and pSNL2, carrying structural genes for L-arabinose utilization were isolated from a *Bacillus subtilis* gene library. Both plasmids complemented *araD* mutations in a Rec⁻ *B. subtilis* strain and in *Escherichia coli*. Moreover, pSNL1 also complemented *araB* mutations in both species and efficiently transformed *araA* Rec⁺ *B. subtilis* strains to Ara⁺. Detailed physical mapping of both plasmids in addition to transformation experiments involving defined restriction fragments from the pSNL1 insert unambiguously determined the gene order to be *araD*, *araB*, and *araA*, an order different from that found in *E. coli*.

The L-arabinose metabolic pathway in *Bacillus subtilis* and its enzymes were described as inducible (6). The three structural genes involved in L-arabinose utilization, *araA*, *araB*, and *araD*, coding for L-arabinose isomerase, L-ribulokinase, and L-ribulose-5-phosphate 4-epimerase, respectively, were mapped between *argA* and *leuA* on the *B. subtilis* chromosome (9; H. Paveia, Ph.D. thesis, University of Lisbon, Lisbon, Portugal).

A gene bank of *B. subtilis* DNA in the shuttle vector pMK4 (10) was screened for the presence of *ara* genes. The gene bank was constructed by partial digestion of the chromosomal DNA from strain QB25 with *Sau*3A followed by insertion of 3- to 6-kilobase (kb) fragments in the single *Bam*HI site of the vector. The total bank represents about 3,000 clones, and the bacterial host used was *Escherichia coli* TG1 (4).

The strains used in this study are listed on Table 1. Plasmid DNA was extracted by the alkaline sodium dodecyl sulfate method (1) from an *E. coli* TG1 culture transformed with pooled DNA from the original clones from the bank in TG1(pMK4). Plasmid DNA was then used to transform competent *B. subtilis* strains with *araA*, *araB*, or *araD* mutations by the method of Bott and Wilson (2), and Ara⁺ clones were selected on minimal C medium plates containing L-arabinose as the sole carbon source (9). With strains IGCg707 (*araB7*) and IGCg724 (*araD24*) several Ara⁺ Cmʳ clones were obtained. Plasmid DNA was isolated from such clones and used to transform competent *E. coli* DH1 cells (8), selecting for ampicillin resistance on LB medium (containing ampicillin at 25 μg ml^{-1}). After transformation of *B. subtilis araA*, *araB*, or *araD* Rec⁺ strains with plasmids isolated from the ampicillin-resistant colonies, only two plasmids, pSNL1 and pSNL2, yielded Ara⁺ Cmʳ transformants. The loss of the *ara* markers from most of the plasmids initially recovered was probably the result of recombination with the chromosome.

Large-scale DNA preparations of pSNL1 and pSNL2 were made by standard methods (7). Physical maps of both plasmids were established by restriction analysis with *Ava*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Kpn*I, *Nco*I, *Pst*I, *Pvu*I, *Sal*I, and *Sma*I (Fig. 1). The enzymes were used as recommended by the suppliers, and DNA fragments were

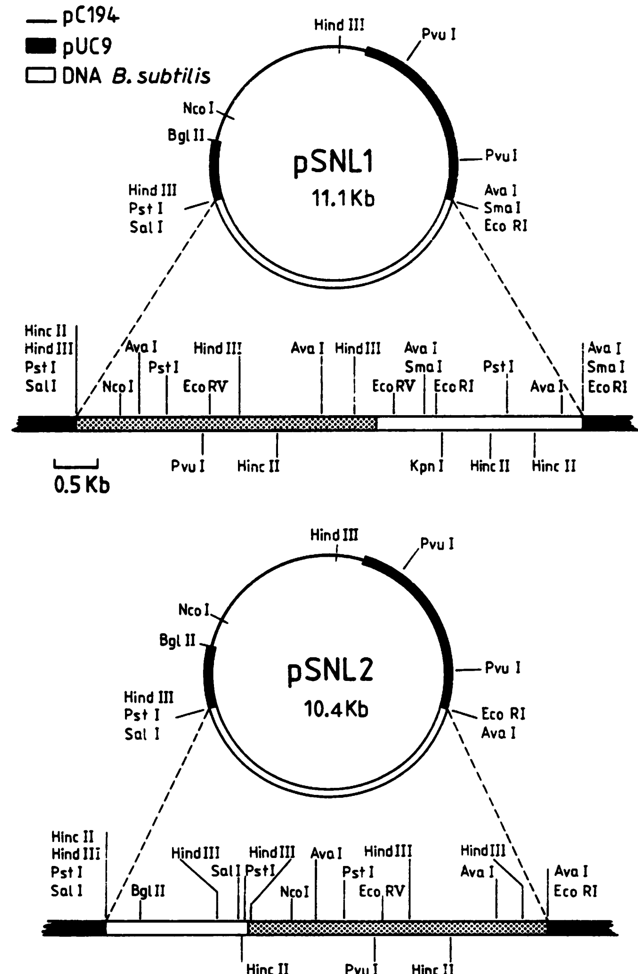

FIG. 1. Restriction endonuclease maps of the cloned DNA inserts of pSNL1 and pSNL2. Maps of the inserts are shown in linear form with specific restriction sites. The shaded region is the cloned region common to pSNL1 and pSNL2.

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TABLE 1. Bacterial strains

Strain	Genotype	Source
B. subtilis		
IGCg707	*araB7 metB10 lys-3*	This laboratory[a]
IGCg717	*araA17 metB10 lys-3*	This laboratory
IGCg724	*araD24 lys-3*	This laboratory
SL1060	*spoIIA69 trpC2 recE4*	P. J. Piggot
IGCg8007	*araB7 lys-3 recE4*	SL1060 transformed into IGCg707
IGCg8017	*araA17 lys-3 recE4*	SL1060 transformed into IGCg717
IGCg8024	*araD24 recE4*	SL1060 transformed into IGCg724
E. coli		
DH1	F^- *recA1 cndA1 gyrA96 thi-1 hsd-817* (r_K^- m_K^-) *supE44 relA1*	P. J. Piggot
TG1(pMK4)[b]	K-12 F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} *lacZ M15* Δ(*lac-pro*) *supE thi hsdD* (pMK4 Amp^r Cm^r)	M. Debarbouillè
SH313	F^- *thr his araC*$^+$ *BA*$^+$*D*$^+$ *dcm galK str*	R. Schleif
RFS855	F^- *thr-1 araA855 leuB6* Δ(*lac*)*74 tsx-274* λ$^-$ *gyrA111 recA11 relA1 thi-1*	R. Schleif via B. Bachmann[c]
RFS54	Hfr *araD54* λ$^-$ *relA1 spoT1 thi-1*	R. Schleif via B. Bachmann

[a] Paveia, Ph.D. thesis.
[b] TG1 has the same genotype but no plasmid.
[c] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

resolved in 0.8 or 1% (wt/vol in Tris-borate buffer) agarose gels.

This analysis showed that inserts of 5.5 and 4.8 kb were present in pSNL1 (11.1 kb) and pSNL2 (10.4 kb), respectively. Furthermore, the constructed restriction maps strongly indicated the occurrence of a 3.5-kb stretch of sequence homology common to both inserts (Fig. 1). This suggestion was confirmed through Southern blot experiments in which ^{32}P-labeled *Hin*dIII fragments of 1.2 and 1.6 kb from pSNL1 were separately used as probes against *Hin*dIII-digested pSNL2. The two complete *Hin*dIII fragments detected are shown in the shaded region of Fig. 1.

To exclude the possibility that the fragment of *B. subtilis* DNA cloned in pSNL1 had suffered any rearrangement in *E. coli*, we compared its structure with that of the corresponding area of chromosomal DNA. This was done by hybridizing the ^{32}P-labeled 4.4-kb *Pvu*I fragment from pSNL1 to total digests of genomic DNA obtained with the various restriction enzymes used to establish the physical map of the cloned fragment. According to our mapping data, there should have been only one band after digestion with *Pvu*I or *Nco*I, two bands after digestion with *Kpn*I, *Pst*I (one of 3.9 kb), *Eco*RI, *Eco*RV (one of 2.1 kb), or *Sma*I, and three bands after digestion with *Hin*dIII (one of 1.2 kb). This pattern was indeed observed (Fig. 2).

Recombination and complementation studies were performed by transforming Rec$^+$ and RecE4 strains bearing mutations in the *araA*, *araB*, or *araD* gene with plasmids pSNL1 and pSNL2. The results (Table 2) indicated that when pSNL1 was used as a donor, Ara$^+$ transformants were obtained with all the recipient Rec$^+$ strains, whereas with pSNL2 transformation was successful only when IGCg724 *araD24* was the recipient. When recombination-deficient *araD24* and *araB7* strains were transformed to Cm^r by pSNL1 most transformants were Ara$^+$, implying positive complementation. Despite the ability to give rise to Ara$^+$ transformants when a Rec$^+$ *araA17* recipient strain was used, pSNL1 was unable to complement a Rec$^-$ strain bearing the same mutation. A low percentage (22%) of Cm^r transformants showed weak growth on C medium supplemented with L-arabinose and chloramphenicol (3 μg ml^{-1}), but after they were restreaked on the same medium, only one clone (presumably an Ara$^+$ or a Rec$^+$ revertant) maintained the Cm^r Ara$^+$ phenotype; this behavior was not further investigated. Plasmid pSNL2 complemented only the *araD24* mutation (Table 2).

When recombination-proficient strains were used, a significant proportion of Cm^r transformants were Ara$^-$, suggesting either gene conversion or plasmid instability. To analyze the mechanism, we chose Ara$^-$ Cm^r transformants

TABLE 2. Transformation of *B. subtilis* strains by plasmids pSNL1 and pSNL2[a]

Recipient strain (relevant genotype)	No. (%) of the indicated transformants obtained with donor plasmid:			
	pSNL1		pSNL2	
	Cm^r	Ara$^+$	Cm^r	Ara$^+$
IGCg707 (*araB7 rec*$^+$)	2.8×10^3 (63)	7.1×10^3 (0)	4.7×10^3	0
IGCg8007 (*araB7 recE4*)	283 (99)	ND	ND	ND
IGCg717 (*araA17 rec*$^+$)	6.6×10^3 (95)	8.9×10^3 (1)	7.9×10^3	0
IGCg8017 (*araA17 recE4*)	210 (22)[b]	ND	ND	ND
IGCg724 (*araD24 rec*$^+$)	6.1×10^3 (79)	3.2×10^4 (0)	2.3×10^3 (83)	3.2×10^3 (0)
IGCg8024 (*araD24 recE4*)	132 (99)	ND	1,591 (97)	ND

[a] Selection was for Cm^r and Ara$^+$, with covalently closed circular plasmid DNA as the donor. Results are reported as the number of transformed colonies per microgram of DNA per milliliter of culture (except with strains IGCg8007, IGCg8017, and IGCg8024, with which data were obtained with 10 μg of DNA per ml of culture) and as the percentage of transformants that also acquired the nonselected Ara$^+$ or Cm^r marker (values in parentheses). ND, Not determined.
[b] See the text.

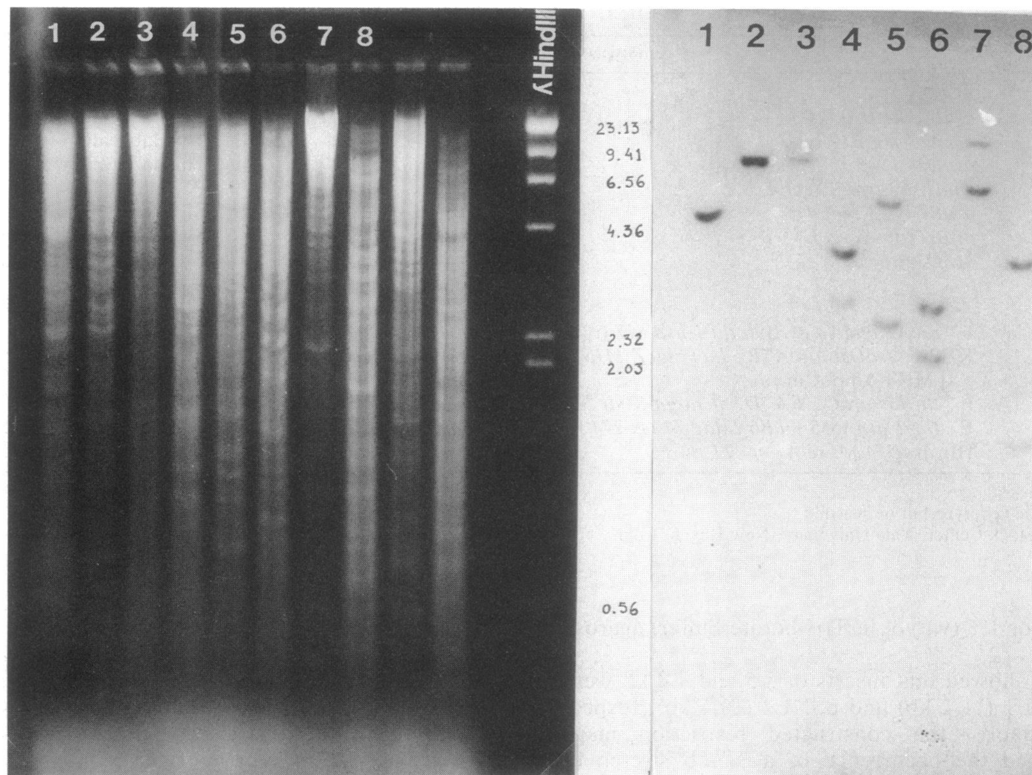

FIG. 2. Correspondence between the inserted DNA of pSNL1 and the chromosomal DNA structure. After total digestion, the restriction fragments were transferred to a nitrocellulose filter and hybridized to a ^{32}P-labeled 4.4-kb *Pvu*I fragment of pSNL1 as the probe. Chromosomal DNA was digested with *Pvu*I (lane 1), *Nco*I (lane 2), *Kpn*I (lane 3), *Pst*I (lane 4), *Eco*RI (lane 5), *Eco*RV (lane 6), *Sma*I (lane 7), and *Hin*dIII (lane 8). The numbers at center indicate the migration of λ *Hin*dIII fragments expressed in kilobases.

from strains IGCg707, IGCg717, and IGCg724 for further study. Plasmids extracted from such clones were digested with *Pst*I and *Eco*RI (or *Sma*I), and their restriction patterns were analyzed in 1% (wt/vol) agarose gels. The plasmids isolated from the five Ara⁻ Cm^r clones generated by pSNL1 in IGCg707 *araA17* had *Pst*I and *Sma*I profiles indistinguishable from the corresponding profiles of pSNL1. Identical results were obtained for the majority of the plasmids isolated from Ara⁻ Cm^r transformants of IGCg707 *araB7*: only 4 plasmids out of a total of 37 examined had a different *Pst*I pattern. Among 21 Ara⁻ Cm^r clones generated by pSNL1 in IGCg724 *araD24*, only 2 contained plasmids with anomalous *Pst*I and *Sma*I restriction patterns (we were unable to isolate plasmids from six of these transformants). Thus, the majority of plasmids did not show gross physical difference from pSNL1. Similar results were obtained by Chak et al. (3) with a plasmid carrying the *spoIIA*$^+$ allele; they obtained occasional recombinants in which the plasmid had acquired the chromosomal mutation. These authors extended to point mutations the observations of Iglesias et al. (5), who showed that deletions and insertions in plasmid-borne chromosomal DNA can be corrected by interaction with the *B. subtilis* chromosomal during transformation. Our results suggest that gene conversion does occur. The differences observed in the proportions of Ara⁻ transformants with the three different strains were similar to those previously observed by Chak et al. (3) and Iglesias et al. (5) and might be the result of the different proportions of monomeric plasmid forms among the different plasmid preparations.

Complementation tests of *E. coli ara* mutations performed with both plasmids produced results essentially equivalent to those obtained with *B. subtilis*, i.e., pSNL2 complementation was restricted to an *araD* mutation, whereas pSNL1 complemented *araB* and *araD* mutations (results not shown).

To ascertain the locations of the *ara* loci in the pSNL1 cloned DNA, we recovered fragments generated by *Ava*I, *Pst*I, and *Hin*dIII (Fig. 3) from the agarose gels by electroelution (7) and used them to transform strains IGCg707, IGCg717, and IGCg724, selecting for Ara$^+$ (Table 3). On the basis of the results we were able to define the gene order as

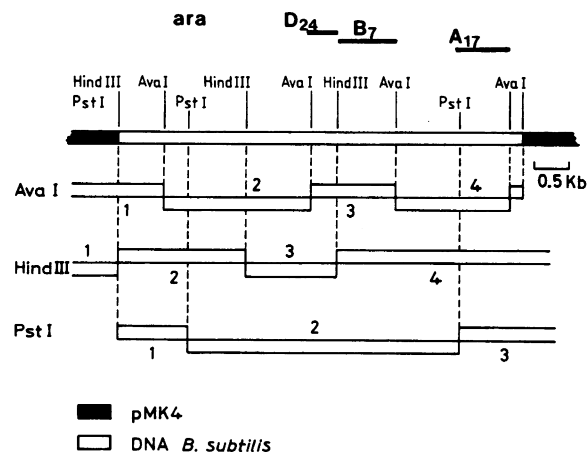

FIG. 3. Locations of the *ara* loci in plasmid pSNL1.

TABLE 3. Transformation of *B. subtilis* Rec$^+$ strains with *ara* mutations, selecting for Ara^{+a}

Recipient	No. of Ara$^+$ transformants obtained with fragment:										
	*Ava*I				*Hin*dIII				*Pst*I		
	1	2	3	4	1	2	3	4	1	2	3
IGCg707 (*araB7*)	1	0	28	0	1	0	1	54	0	137	0
IGCg717 (*araA17*)	2	2	2	342	3	0	0	1,360	1	0	87
IGCg724 (*araD24*)	0	0	932	1	1	0	44	0	0	432	20

[a] Several fragments generated by digestion of pSNL1 with *Ava*I, *Hin*dIII, or *Pst*I (Fig. 3) were recovered from the agarose gels and used to transform *B. subtilis* Rec$^+$ strains. The fragment numbers correspond to those shown in Fig. 3. The concentrations of DNA were not determined.

araD, *araB*, and *araA*, an order different from that found in *E. coli*: *araB*, *araA*, and *araD*.

The location of the *ara17* locus at one end of the cloned fragment, together with the absence of *araA* complementation with pSNL1, suggests that only part of the *araA* gene has been isolated. Experiments aimed at cloning the chromosomal DNA region adjacent to the one present in pSNL1 are currently in progress.

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