JOURNAL OF BACTERIOLOGY, July 1982, p. 521–523
0021-9193/82/070521-03$02.00/0

Vol. 151, No. 1

Complementation of the *leuB6* Allele of *Escherichia coli* by Cloned DNA from *Mucor racemosus*†

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Received 19 October 1981/Accepted 11 February 1982

A recombinant plasmid, designated pMu *leu1*, was constructed from *Mucor racemosus* genomic DNA and *Escherichia coli* plasmid pBR322. This plasmid complemented the *leuB6* mutation of *E. coli*, apparently by suppression. The plasmid contained two *Hin*dIII fragments of approximately 3.0 and 1.7 kilobases. Neither fragment alone exhibited complementing activity. Several proteins were specified by the plasmid, but their role, if any, in complementation is unknown.

Chimeric plasmids constructed from the DNA of certain lower eucaryotic organisms and a suitable vector may complement mutations in auxotrophic strains of *Escherichia coli* (11, 13). Such plasmids have been isolated by first preparing a gene bank of the genome of the organism and then introducing the cloned DNA into the bacterial cell. Subsequent selection for growth on a medium lacking the required nutrient can lead to the tentative identification of bacterial colonies harboring a recombinant plasmid that complements the gene in question. This approach has resulted in the isolation of a number of genes from *Saccharomyces cerevisiae*, among them sequences encoding the *ura1* (1), *trp1* (14), *leu2* (11), and *arg4* (3) gene products. In a like manner, the *qa-2* gene of the quinic acid catabolic pathway in *Neurospora crassa* (15) and the structural gene for β-galactosidase from *Kluyveromyces lactis* (16) have been isolated.

In addition to direct complementation of such mutations by the apparent functional expression of the corresponding gene product from another species, the putative suppression of bacterial mutations has been noted after introduction of *S. cerevisiae* DNA into a *leuB6* auxotroph of *E. coli* (11). We recently observed a similar occurrence by using cloned genomic DNA of the fungus *Mucor racemosus* and report the results here.

In these experiments, groups of 250 members were assembled from a *Mucor* clone bank maintained in *E. coli* plasmid pBR322 (2). Crude lysates were prepared, and plasmid pools were isolated by standard techniques (4). The individual pools were then introduced into several amino acid-requiring *E. coli* strains by transformation (5) and plated onto the appropriate medium. Putative transformants occurred only for the *leuB* marker, and this event only occurred with plasmids from a single pool.

Several transformants were chosen, and the plasmids from them were purified for further analysis. Upon digestion with restriction endonuclease *Hin*dIII and subsequent electrophoresis of the digests, two fragments of approximately 3.0 and 1.7 kilobases were observed in all cases (Fig. 1A, lane 2). In addition, three *Xho*I, two *Eco*RI, and one *Xba*I restriction endonuclease sites were observed on the insert. No *Bam*HI or *Pst*I sites were noted. A hybridization probe was prepared from the plasmid, designated pMu *leu1*, by nick-translation (8), followed by hybridization of the probe to *Hin*dIII-digested *Mucor* DNA. These experiments showed that identical fragments resided within the genomic DNA (Fig. 1B).

Additional experiments were performed to determine whether pMu *leu1* could confer the ability to grow in the absence of leucine for other nonallelic leucine auxotrophs. Transformants arose only for strains with a *leuB6* mutation (Table 1). No growth occurred in *E. coli* strains with either a different *leuB* lesion or a mutation in the *leuA* gene. Similar attempts to complement the equivalent gene (*leu2*) in *S. cerevisiae* were unsuccessful.

Since pMu *leu1* complemented only the *leuB6* allele of the *leuB* gene, it seemed unlikely that the plasmid contained sequences that directed the biosynthesis of isopropylmalate dehydrogenase, the *leuB* product. Instead, it seemed more likely that sequences on pMu *leu1* suppressed the mutation in a manner analogous to the phenomenon mediated by cloned *S. cerevisiae* DNA, as mentioned above (11). Attempts were made to identify the nature of the presumed suppressing activity as follows. (i) The two *Hin*dIII fragments comprising pMu *leu1* were

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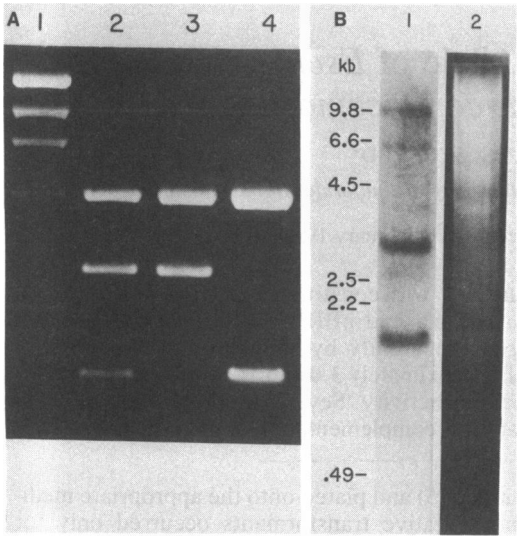

FIG. 1. (A) Electrophoretic separation of pMu *leu1* and subcloned fragments. Plasmids were digested with *Hin*dIII and subjected to electrophoresis in a 1.2% agarose gel. Lane 1, λ; lane 2, pMu *leu1*; lane 3, subcloned 3.0-kilobase *Hin*dIII fragment; lane 4, subcloned 1.7-kilobase *Hin*dIII fragment. (B) Hybridization of nick-translated pMu *leu1* to *Hin*dIII-digested *Mucor* DNA. Lane 1, Autoradiograph after hybridization of pMu *leu1* to genomic DNA; lane 2, *Hin*dIII digestion of *Mucor* DNA. Nick-translation and blotting techniques were performed as described elsewhere (12).

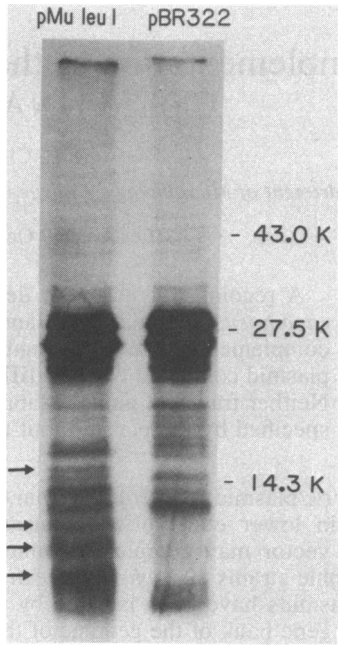

FIG. 2. Minicells were purified from strain P678-54 essentially as described previously, with minor modifications (9). Sucrose gradients were prepared by the freeze-thaw of a 20% sucrose solution. After starvation for methionine in M9 medium containing methionine assay medium (1:4) for 60 min, proteins were labeled with [^{35}S]methionine to a concentration of 50 nCi/ml for 30 min. One-dimensional electrophoresis was by the method of Laemmli (7).

subcloned (Fig. 1), and the resulting plasmids were introduced into *E. coli* JA221 or CR34, each bearing the *leuB6* mutation. Neither fragment alone was capable of conferring the ability to grow in the absence of leucine on either strain, indicating that the internal *Hin*dIII site of pMu *leu1* was within a region whose integrity was required for suppression to occur. (ii) No sequences homologous to *Mucor* tRNA species were present on the fragment, as judged by the lack of hybridization of ^{32}P-labeled total *Mucor* tRNA to any portion of pMu *leu1*. (iii) Three proteins exhibiting apparent molecular weights of 10,000, 11,000, and 12,000, respectively, could be detected in minicells when protein synthesis was directed by pMu *leu1*, but not when synthesis was directed by pBR322 alone (Fig. 2). A larger protein of 16,500 daltons was also observed in most experiments, but in smaller quantities than the other species. It was not clear whether each protein was translated independently or they occurred as a result of sequential degradation of a larger primary product. Pulse-chase experiments failed to resolve this question. However, biosynthesis of the 11,000-dalton protein occurred when protein synthesis was directed by the 1.7-kilobase *Hin*dIII subcloned fragment (data not shown), indicating that this protein species was not a degradation

TABLE 1. Transformation of *E. coli* auxotrophs with pMu *leu1*

E. coli strain[a]	Allele tested	Transformant appearance[b] on minimal medium (days)	Amp$^+$ Leu$^+$ transformants per μg of DNA
JA221	*leuB6*	8–10	7.5×10^4
CR34	*leuB6*	20–25	5.2×10^4
CV438	*leuB061*	—[c]	0
CV437	*leuA371*	—	0

[a] Strain JA221 was received from John Carbon. CR34 was a C600 derivative and carried the *supE* mutation (10). Strains CV438 and CV437 were from J. Calvo.

[b] The indicated strains were transformed by routine methods (3). Minimal medium was M9 supplemented with the appropriate requirements. Incubation temperature was 34°C. The efficiency of transformation was tested in all cases by plating samples of cells on L-agar supplemented with 100 μg of ampicillin per ml.

[c] —, No transformants detected after 30 days of incubation.

product. The 10,000- and 12,000-dalton pMu *leu1*-specified proteins were not observed in minicells containing either of the subcloned *Hin*dIII fragments. Therefore, the possibility remains that one of the observed proteins is directly responsible for the complementing activity.

In summary, we have observed the apparent suppression of the *leuB6* mutation in *E. coli* with cloned *Mucor* DNA. It is intriguing that complementation by suppression of this mutation apparently can also be mediated with several recombinant plasmids constructed with *S. cerevisiae* DNA (11). The exact mechanism of the suppression remains unknown, and its elucidation will depend in part on a thorough study of the *leuB6* mutation itself.

We thank Carol Katayama for expert technical assistance.

This work was supported in part by Public Health Service grants GM 26690 and GM 23999 from the National Institutes of Health (NIH). R.L.C. was the recipient of a Public Health Service postdoctoral National Research Service Award from the NIH.

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