PLASMID pVA517C FROM *ESCHERICHIA COLI* V517 IS REQUIRED FOR THE EXPRESSION OF AN ANTIBIOTIC MICROCIN

Sir:

Escherichia coli V517 is a citrate utilizing clinical isolate, widely used in many laboratories as a source of eight different plasmids of known molecular weight for agarose gel electrophoresis[1]. So far, no phenotype has been ascribed to any of these plasmids, even the citrate-utilizing ability. This phenotype is uncommon in *E. coli*, and it has been shown that is plasmid born in other *E. coli* strains[2].

In this paper we demonstrate that plasmid pVA517C from *E. coli* V517 is responsible for the production of and the resistance to one antibiotic of the microcin group. Microcins are low molecular antibiotics produced by different genera of Enterobacteriaceae, mainly *E. coli*[3~5]. Most of the microcin-producing strains have been classified in four groups, corresponding to different types of activities (F. SÁNCHEZ *et al.* manuscript in preparation). It has been also observed that microcin synthesis is encoded by plasmid in most of the cases so far studied[6~8]. In all microcinogenic strains tested, it has been possible to associate each type of activity with a single plasmid (F. SÁNCHEZ *et al.* manuscript in preparation).

The production of a microcin by *E. coli* V517 was detected as described[3], using either strains *E. coli* B or *E. coli* 405 as lawns. After incubation at 37°C for 18 hours an inhibition halo was observed centered by the spots of V517. The inhibition halo was also produced when a dialysis membrane was placed between strain V517 and the strain used as a lawn, suggesting that inhibition was due to an antibiotic of low molecular weight and not to the presence of colicins. A partially purified microcin preparation was obtained from supernatants of cultures of *E. coli* V517 as described[9], indicating a close similarity to the group A of microcins. The best studied example is microcin A15, formerly named 15m[9,10].

In order to determine if one of the plasmids present in *E. coli* V517 was responsible for microcin activity, co-transformation experiments were performed. The plasmid pPK200, which is a mutant derivative thermo-unstable in replication, of plasmid pSC101[11] was used. It was possible to select transformants at 32°C by means of the tetracycline resistance gene carried by pPK200. Subsequently, the transformants were cured of plasmid pPK200 by incubation of the cells at 42°C. Similar, but less reproducible, results were obtained by direct selection with purified microcin as described in ref 7.

The purification of plasmid DNA from pPK200 and from the eight plasmids of *E. coli* V517 was carried out from 250 ml cultures grown in L broth[12], according to BIRNBOIM and DOLY[13] with minor modifications. Further plasmid purification was achieved by ultracentrifugation in cesium chloride-ethidium bromide gradients. After collecting the band corresponding to CCC DNA under UV light, the dye was extracted with isoamyl alcohol, and the solution containing plasmid DNA dialyzed against TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). At this stage plasmid DNA was used in co-transformation experiments. Transformation of *E. coli* HB101 was performed as described by KRETSCHMER *et al.*[11]. One microgram of mixture of the eight different plasmids of *E. coli* V517 was mixed with 10 to 100 ng of plasmid pPK200 in three different experiments.

Selection for tetracycline resistant colonies (12 μg/ml) was at 32°C. These transformants were scored for their ability to produce microcin A15-like[9] and then grown at 42°C to cure pPK200. The number of transformants selected by resistance to tetracycline were between 10^4 to 10^5 transformants per microgram of pPK200 plasmid DNA. The frequencies of co-transformation of microcin clones was close to 10% in 600 clones examined.

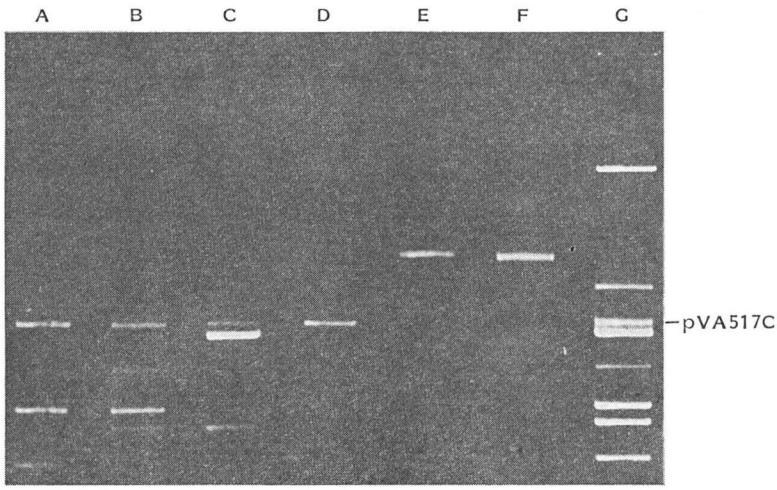
Plasmid DNA was isolated from the transformants and all the transformants that were microcin producers harbored a 5.5 kb plasmid, indistinguishable by size to plasmid pVA517C from *E. coli* V517 (Fig. 1). In addition, the transformants that did not produce microcin activity lacked pVA517C (data not shown). These results indicate that microcin A15-like activity of *E. coli* V517 is dependent upon the presence of plasmid pVA517C. Experiments directed to establish whether the microcin produced by *E. coli* V517 is identical to microcin A15 are in progress. We are also studying if there exists any homology between pVA517C and pCP101 from *E. coli* LP15, the original

Fig. 1. Gel electrophoresis of the plasmids.

Agarose gel electrophoresis (0.7%) of plasmid DNA, purified as described in the text.

Lanes A, B, C and D correspond to four different co-transformants with pPK200 and DNA from *E. coli* V517, all of them were microcin A15-like producers. Lane E: pSC101, lane F: pPK200. Lane G corresponds to *E. coli* V517 plasmid DNA.

Purification of plasmid DNA from the microcinogenic transformant clones was carried out after growth at 42°C to eliminate pPK200. Electrophroresis was at 120 V for 2 hours in 90 mM Tris, 90 mM boric acid and 2.5 mM EDTA.



microcin A15 producer strain[7].

Acknowledgments

We thank F. CABELLO for the supply of plasmid pPK200 and suggestion of co-transformation experiments, and MARIA LORENZO for technical assistance. We thank the Plasmid Reference Center (Stanford University) for providing us with *E. coli* V517.

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(Received February 28, 1986)

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