Communications to the editor

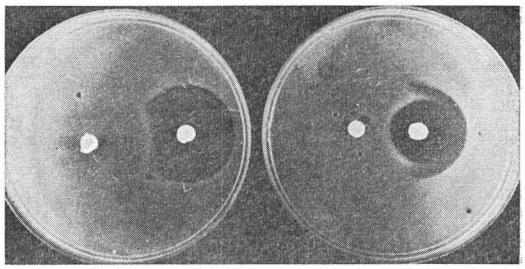
MICROCIN 15n: A SECOND ANTIBIOTIC FROM *ESCHERICHIA COLI* LP15

Sir:

Microcins are low molecular weight antibiotics produced by *Escherichia coli* strains[1], and their synthesis is coded by plasmids in most of the cases so far studied[2]. One of these strains, *E. coli* LP15, produces microcin 15m which inhibits the first enzyme of the methionine biosynthetic pathway, namely homoserine-*O*-transsuccinylase[3,5]. This work reports the finding of a second antibiotic activity in the supernatants of cultures of LP15. This antibiotic is not antagonized by common amino acids, bases or vitamins and consequently shows activity in rich media. The existence of this new antibiotic, which is hereafter named microcin 15n, could be inferred from the absence of lack of methionine antagonism. Inhibition zones around dense spots of *E. coli* LP15 on sensitive strains did not disappear in the presence of methionine. It was also found that crude preparations of microcin 15m behave anomalously in regard to L-methionine antagonism. These observations led to the detection of a second antibiotic activity, which can be visualized in the photograph (right plate) of Fig. 1. *E. coli* cultures and concentrates (500-fold) were prepared as described previously[1]. The separation of microcin 15n from microcin 15m was achieved by applying a concentrate (500-fold) of microcins (15m and 15n) to a colum of Dowex 50WX4(H), equilibrated at pH 2.5. In contrast to microcin 15m[3], microcin 15n activity was not retained on the column and eluted when the column was washed with HCl, pH 2.5. The active fractions were quickly neutralized with 2 N NH_4OH and pooled. This partially purified product was used for a preliminary study of the properties of this microcin and for determining its antimicrobial spectrum. Microcin 15n has a molecular weight of about 500, as assayed by filtration through Amicon and Pellicon filters and gel filtration through Sephadex G-10 and G-15; it is relatively thermoresistant (100°C, 30 minutes), soluble in methanol - water (5: 1), but not in methanol 100%; it is not adsorbed on activated charcoal or hydrophobic matrices like Bondapack C_{18}. It is sensitive to extreme pH values, as well as to the action of proteases (pronase, subtilisine, trypsin and proteinase K) which quickly destroy its activity. It is also inactivated by oxidative agents such as hydrogen peroxide. These assays were performed as described previously[1], and suggest that the microcin has, at least in part, a peptide structure. It seems to be a polar molecule according to the chromatographic and solubility data and, apparently, has a low molecular weight.

Fig. 1. Detection of microcin 15n produced by *E. coli* LP15.

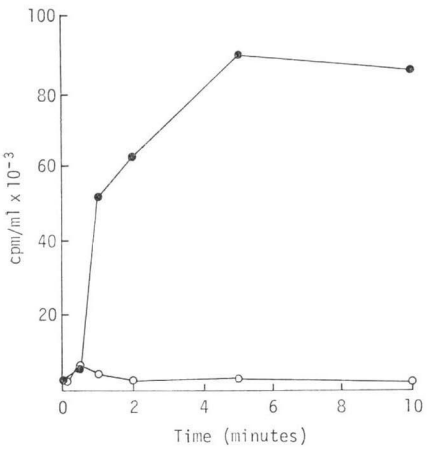
The disk on the left in both plates contained 0.5 mg of L-methionine and that on the right 50 μl of a crude preparation (500-fold) of microcins (15m and 15n). In the plate of the right, where the disks are closer, a halo of inhibition, not antagonized by L-methionine, can be visualized.



To study the mechanism of action of microcin 15n, the incorporation of labelled precursors into proteins, DNA and RNA was studied using *E. coli* 405 as a test strain. These assays were performed as described before[3]. In every case, a rather abrupt inhibition was observed (data not shown). These data do not necessarily indicate a direct action of microcin 15n on the mechanism involved in the incorporation of these metabolites into macromolecules. On the contrary, this apparent unspecificity points to the fact that these inhibitions could be the consequence of the action at other levels. There are some preliminary evidence that the primary target of microcin 15n is the energy-generating system of the cell. Thus, the uptake of labelled glucose by *E. coli* 405 is greatly inhibited as it can be seen in Fig. 2. The uptake measurements were carried out as describ-

Fig. 2. Inhibition of D-[U-^{14}C]glucose uptake in *E. coli* 405 by microcin 15n.

A mid-exponential culture of *E. coli* 405 grown at 37°C was divided into two tubes each containing 5 ml, and the temperature was mantained constant at 37°C. To the first tube 0.35 ml of microcin 15n was added with a specific activity of 800 AU/ml. Then, 20 μCi of D-[U-^{14}C]glucose was immediately added to each tube. At different times aliquots of 50 μl were filtered through Millipore filters (pore size 0.45 μm), the cells were washed on the filters with 10 mM tris-HCl, pH 7.3, 0.15 M NaCl and 0.5 M $MgCl_2$. The radioactivity of the filters, once dried, was measured in a scintillation counter.



ed by DURO *et al.*[4] Nevertheless, microcin 15n does not lyse sensitive cells, even at concentrations ten-fold higher than that necessary to inhibit cellular growth.

The antibacterial spectrum of microcin 15n is presented in Table 1. It can be seen that microcin 15n is mainly active against *Enterobacteriaceae*, with little activity on other genera (data not shown). These data have to be considered qualitative since pure microcin 15n is not available yet in large enough quantity.

This microcin has been shown to be coded for by a plasmid of 3.7 Mdalton and transconjugants have been obtained that produce only microcin 15n and not the two antibiotics as in wild type *E. coli* LP15 (F. SÁNCHEZ, *et al.*, manuscript in preparation). Studies on the kinetics of microcin production made by these transconjugants showed the presence of a sharp peak of activity at the end of the exponential growth phase followed by a rapid decay concomitant with the beginning of the stationary phase. This decay is common to the production kinetics of other microcins, and

Table 1. Activity of microcin 15n against *Enterobacteriaceae*.

Microorganism[a,b]	Mean inhibition zone (mm)
Escherichia (6)$^+$	18
Citrobacter (2)	27
Klebsiella (4)$^+$	18
Enterobacter (2)	15
Proteus (5)	20
Salmonella (7)	17
Shigella (3)	16

[a] The numbers in brackets indicate the number of strains assayed. The numbers on the right of each genus represent the average of the diameter of the inhibition zones produced by microcin 15n on each of the species assayed. For each assay, 20 μl of microcin 15n with an activity of 800 AU/ml were added on top of sterile paper disks. AU (arbitrary units) is defined as the minimal amount of microcin 15n that produces a visible inhibition halo on a lawn of *E. coli* 405 grown on solid minimal medium.

[b] Strains marked (+), although sensitive to the microcin preparation used, were not always sensitive to the producing strain in spot tests (F. SÁNCHEZ, personal communication).

in some cases it could be due to the excretion of a microcin-antagonist by the producing strain (V. DE LORENZO, *et al.*, manuscript in preparation).

Acknowledgements

We are indebted to Drs. J. F. MARTÍN, V. DE LORENZO and J. F. GARCÍA-BUSTOS for their criticism and valuable suggestions. This work was supported by the Plan Concertado 3/76 of the Comisión Asesora de Investigacion Científica y Técnica and by the Fondo de Investigaciones Sanitarias, Ministerio de Sanidad y Seguridad Social.

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(Received October 4, 1982)

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