

Extrachromosomal Nature of Hydrogen Sulfide Production in *Escherichia coli*

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Received for publication 2 March 1971

Two strains of *Escherichia coli* which produce hydrogen sulfide appear to have acquired this ability via transfer of genetic material from another genus.

The ability of some clinically derived *Salmonella* (1) and *Proteus* (4, 8) strains to utilize lactose has been shown to reside in extrachromosomal deoxyribonucleic acid (DNA) that exhibits a base composition different from that of the host DNA. In addition, the *lac* character can be transferred from these strains to related strains.

This report deals with two strains of *Escherichia coli*, PU1 and PU2, which can produce hydrogen sulfide. Strains PU1 and PU2 were derived from clinical sources and were submitted to the Center for Disease Control for definitive identification. Aside from the ability to produce H₂S, both strains exhibited biochemical and serological properties characteristic of *E. coli* (2, 3). H₂S production was routinely checked in SIM agar (Difco).

For the preparation of DNA, the cells were grown in liquid culture, and aeration was effected by means of a shaker-incubator at a temperature of 30 C. One liter of culture medium contained: 14 g of K₂HPO₄, 6 g of KH₂PO₄, 2 g of (NH₄)₂SO₄, 0.2 g of MgSO₄·7H₂O, and 10 g of glucose. The cells were harvested in mid-log phase and used for the preparation of DNA by the method of Marmur (5). Isopycnic density gradient sedimentation in CsCl was performed in a Spinco model E ultracentrifuge equipped with ultraviolet optics. The fractionation of the DNA on columns of methylated albumin and Kieselguhr (MAK) was performed by the procedure of Sueoka and Cheng (7).

Strain PU2 was observed to segregate H₂S⁻ colonies spontaneously at a frequency of approximately 1%, whereas PU1 showed a much lower rate of spontaneous segregation of the H₂S character. Neither ethidium bromide nor acridine orange significantly affected the rate of segregation. Attempts to demonstrate the transfer of H₂S production into laboratory strains of *E. coli* were hindered by the lack of an effective

screening procedure, and we can only state that the transfer, if it occurred, was effected at a frequency much less than 1%.

The DNA of strain PU1 appeared to be of

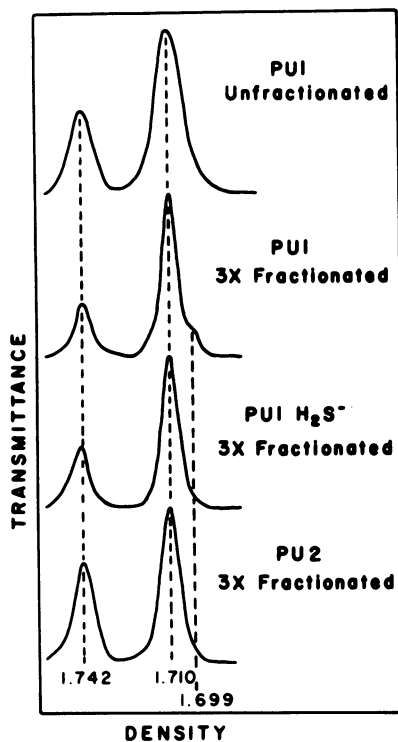


FIG. 1. Densitometer tracings of photographs of the isopycnic centrifugation of DNA in a gradient of CsCl. Fractionations of the DNA were performed on MAK columns. The CsCl solution contained 2 μ g of *E. coli* DNA per ml and 1 μ g of ¹⁵N-DNA from *Pseudomonas aeruginosa* per ml which was used as a standard. The solutions were centrifuged at 44,770 rev/min for 22 hr at 25 C. The peak at the far left represents the ¹⁵N-DNA of *P. aeruginosa*.

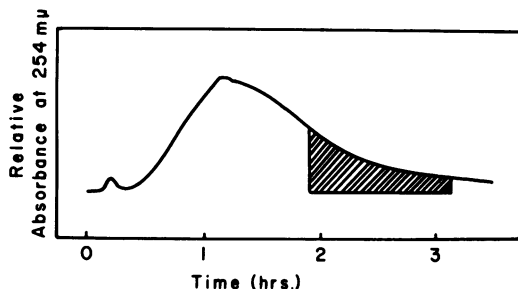


FIG. 2. Elution profile of the chromatography of DNA on a column of MAK. The relative absorbance at 254 nm (by ISCO ultraviolet monitor) is shown. The crosshatching indicates the portion of the elution curve taken for refractionation or ultracentrifugation.

TABLE 1. Buoyant density and per cent guanine plus cytosine of PU strains

Source of DNA	Buoyant density	Guanine + cytosine (%)
PU1 parental	1.709 - 1.711	50 - 52
PU1 satellite	1.698 - 1.700	39 - 41
PU2 parental	1.709 - 1.711	50 - 52
<i>Escherichia coli</i> (literature)	1.710 ^a	51 ^a
<i>Proteus mirabilis</i>	1.700 ^a	41 ^a
<i>P. vulgaris</i>	1.700 ^a	41 ^a

^a Data from Schildkraut, Marmur, and Doty (6).

uniform density until it was subjected to fractionation on MAK (Fig. 1). A typical elution profile of the fractionation of the DNA on an MAK column is shown in Fig. 2. It is clear from Fig. 1 that a satellite band of DNA can be demonstrated after three passages of the DNA through the MAK column. Further, DNA from an H₂S⁻ segregant of PU1 showed no trace of a satellite band after similar fractionation on MAK. A similar examination of the DNA from strain PU2 did not reveal the presence of a satellite band.

The per cent guanine plus cytosine of the DNA bands in Fig. 1 was calculated from the positions of the bands by the method of Schildkraut et al. (6). As is evident from Table 1, the per cent guanine plus cytosine of the satellite band of PU1 DNA was very close to the value reported from *Proteus mirabilis* and *P. vulgaris* (6).

The observation of a satellite band of DNA in strain PU1 suggests that H₂S production in *E. coli* may be the result of the introduction of an extrachromosomal genetic factor from another genus, possibly *Proteus*. Our inability to demonstrate a corresponding satellite band of DNA in strain PU2 probably reflects a smaller amount of the extrachromosomal DNA in that strain.

We thank C. H. Grunwald of the Department of Agronomy, University of Kentucky, for his help in obtaining the densitometer tracings shown in Fig. 1.

LITERATURE CITED

- Easterling, S. B., E. M. Johnson, J. A. Wohlhieter, and L. S. Baron. 1969. Nature of lactose-fermenting *Salmonella* strains obtained from clinical sources. *J. Bacteriol.* **100**:35-41.
- Edwards, P. R., and W. H. Ewing. 1962. Identification of enterobacteriaceae, 2nd ed. Burgess Publishing Co., Minneapolis.
- Ewing, W. H. 1970. Enterobacteriaceae: biochemical methods for group differentiation. Public Health Service publication no. 734, U. S. Government Printing Office, Washington, D. C.
- Falkow, S., J. A. Wohlhieter, R. V. Citarella, and L. S. Baron. 1964. Transfer of episomic elements to *Proteus*. II. Nature of *lac*⁺ *Proteus* strains isolated from clinical specimens. *J. Bacteriol.* **88**:1598-1601.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **3**:208-218.
- Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* **4**:430-443.
- Sueoka, M., and T. Y. Cheng. 1962. Fractionation of nucleic acids with the methylated albumin column. *J. Mol. Biol.* **4**:161-172.
- Wohlhieter, J. A., S. Falkow, R. V. Citarella, and L. S. Baron. 1964. Characterization of DNA from a *Proteus* strain harboring an episome. *J. Mol. Biol.* **9**:576-588.