JOURNAL OF BACTERIOLOGY, Aug., 1967, p. 486-487
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Vol. 94, No. 2
Printed in U.S.A.

Deoxyribonucleic Acid Homologies Among Some Bacteria

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Received for publication 17 February 1967

The deoxyribonucleic acid (DNA)-agar method has been employed to demonstrate homologies among the DNA's of several members of the *Enterobacteriaceae* (B. J. McCarthy and E. T. Bolton, Proc. Natl. Acad. Sci. U.S. **50**:156, 1963). This work used the extent of reaction (binding) of radiolabeled DNA fragments to a series of DNA-agars as the index of polynucleotide homology. Competition experiments, in which a large excess of unlabeled DNA fragments competed with labeled fragments for homologous sites on the agar-immobilized DNA (B. H. Hoyer, B. J. McCarthy, and E. T. Bolton, Science **144**:959, 1964), have also been used to demonstrate interspecies homology.

The present report directly compares these two experimental approaches. The data obtained from binding and competition experiments are shown in Table 1. Specificity of the technique was assured by control experiments in which the background binding of *Escherichia coli* B fragments and *Salmonella typhimurium* strain LT2 fragments to agar containing no DNA was 0.4 and 0.25%, respectively. In addition, DNA fragments from *Brucella neotomae*, an organism unrelated to the *Enterobacteriaceae*, were unable to compete with *E. coli* fragments for sites on *E. coli* DNA-agar (Table 1, bottom line).

TABLE 1. *Relatedness of the DNA of Escherichia coli, Salmonella typhimurium, and Aerobacter aerogenes*

Source of DNA in agar	Source of ^{32}P-labeled fragments	Source of unlabeled competitor fragments	Per cent relatedness at 60 C	No. of experiments	Per cent relatedness at 66 C	No. of experiments
E. coli B	*S. typhimurium* LT2	None	34	8	26	6
S. typhimurium LT2	*E. coli* B	None	38	2	—	—
E. coli B	*E. coli* B	*S. typhimurium* LT2	39	5	—	—
E. coli B	*E. coli* B	*S. typhimurium* 7823	48	3	—	—
E. coli B	*E. coli* B	*A. aerogenes*	41	3	—	—
E. coli B	*E. coli* B	*Brucella neotamae*	0	1	0	1

[a] Either 0.25 or 0.5 g of agar was incubated for 16 hr at 60 or 66 C with an equal volume of labeled DNA fragments in 2 × SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate). In competition experiments, unlabeled DNA fragments were included in the reaction mixture. The ratio of DNA in the agar to labeled DNA fragments was between 200:1 and 1,000:1. The agars were held at the incubation temperature and washed with 10 successive 15-ml portions of 2 × SSC (15 min each) to remove nonhybridized fragments. Three successive washes in distilled water at 75 C were used to elute the hybridized fragments. The wash solutions were precipitated with 5% trichloroacetic acid in the presence of 50 μg of RNA carrier, filtered, and assayed for radioactivity. Homologous binding was 23 ± 4% at 60 C and 18 ± 5% at 66 C. In binding experiments, interspecies relatedness is expressed as percentage of heterologous fragments bound when the homologous binding is normalized to 100%. Relatedness in competition experiments is expressed as (per cent binding − per cent binding in presence of heterologous competitor)/(per cent binding − per cent binding in presence of homologous competitor) × 100.

At 60 C, the incubation temperature normally employed, 34% homology of ^{32}P-labeled *S. typhimurium* strain LT2 fragments was demonstrated with *E. coli* DNA-agar. In the reciprocal experiment, 38% homology of the ^{32}P-labeled *E. coli* DNA fragments was demonstrated with *S. typhimurium* strain LT2 DNA-agar. Comparable results (39% homology) were obtained from competition experiments in which *S. typhimurium* strain LT2 fragments served as competitor against the binding of labeled *E. coli* fragments to *E. coli* DNA-agar.

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A second *S. typhimurium* strain (7823) and a laboratory strain of *Aerobacter aerogenes* were shown to be 48 and 41% related to *E. coli* B on the basis of competition experiments. The value for *Aerobacter* homology is in good agreement with the 45 and 51% homology previously reported (B. J. McCarthy and E. T. Bolton, Proc. Natl. Acad. Sci. U.S. **50**:156, 1963) for binding of two *Aerobacter* strains to *E. coli* DNA-agar. However, the homology observed between *E. coli* and *S. typhimurium* strains LT2 and 7823 is significantly lower than the 71% value for *E. coli* B and *S. typhimurium* strain 7823 also reported by McCarthy and Bolton. Independent studies (S. Falkow, *personal communication*) support a value of approximately 35% homology between the DNA of *E. coli* and those of a number of *S. typhimurium* strains as determined by binding experiments (strain 7823 was not tested).

It should be emphasized that the extent of homology reported is valid only for reactions carried out at 60 C in 2 × SSC (standard saline citrate). Animal DNA is known to exhibit a decrease in both intraspecies and interspecies binding and an increase in discrimination power (ratio of intraspecific binding to interspecific binding) with increasing incubation temperature (M. A. Martin and B. H. Hoyer, Biochemistry **5**:2706, 1966). Preliminary experiments indicate similar results with bacterial DNA. The amount of both intra- and interspecies binding fell when the incubation temperature was raised from 60 to 66 C, and the degree of homology between *S. typhimurium* strain LT2 and *E. coli* B fell from 34% at 60 C to 26% at 66 C.