Deoxyribonucleic Acid Hybridization Among Some Species of the Genus *Pasteurella*

DANIEL B. RITTER AND ROBERT K. GERLOFF

Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, Hamilton, Montana

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The technique of measuring deoxyribonucleic acid (DNA) complementarity described by B. J. McCarthy and E. T. Bolton (Proc. Natl. Acad. Sci. U.S. 50:156, 1963) has enabled genetic comparisons of various bacteria, viruses, mammals, and other organisms. DNA homologies existing among five species of bacteria now classified in the genus *Pasteurella* (Bergey's Manual, 7th edition) were studied. The species were: *P. tularensis* (*tul*), Jap strain; *P. novicida* (*nov*), strain U-112; *P. pestis* (*pes*), strain 19SP; *P. pseudotuberculosis* (*psTB*), strain 344-14; *P. multocida* (*mult*), strain 413BK. Also included were *Escherichia coli* (*Ec*), strain B, from the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N.Y., and an unidentified bacterial isolate (*Ui*), CRO 319-031. The latter was representative of several isolates, from lake water and muskrats collected in Utah in 1960, which possessed characteristics in common with species of the genus *Pasteurella* (C. R. Owen, personal communication).

Brucella broth (Albimi Laboratories, Inc., Flushing, N.Y.) was used to grow *psTB* (24 hr) and *mult*, *pes*, and *Ui* (40 hr). A broth of pH 6.8 containing 2% Bacto-Peptone, 0.1% glucose, 0.1% cysteine, and 2% rabbit serum was used to grow *tul* and *nov* (24 hr). *Ec* was cultured for 24 hr in M-9 broth (R. B. Roberts et al., Carnegie Inst. Wash. Publ. 607, p. 5, 1955) supplemented with 0.5% glucose. All cultures were incubated on a shaker at 37 C for the above-mentioned intervals, selected to give maximal yields of DNA. For production of radiolabeled DNA, 0.005 to 0.01 mc of $P^{32}O_4$ per ml was added to the specified medium. DNA was extracted and purified by the method of J. Marmur (J. Mol. Biol. 3:208, 1961) and fragmented by the method of McCarthy and Bolton (1963) as modified by Gerloff et al. (J. Infect. Diseases 116:197, 1966). The average molecular weight of the fragmented DNA was 2.5×10^5 . Labeled DNA preparations had activities averaging 25,000 counts per min (range, 4,000 to 54,000) per μ g of DNA, determined with a Packard Tri-Carb spectrometer.

Native DNA was embedded in agar essentially as described by E. T. Bolton and B. J. McCarthy (Proc. Natl. Acad. Sci. U.S. 48:1390, 1962). The average quantity of DNA immobilized in agar was 765 μ g per g of agar (range, 457 to 964 μ g). Embedded DNA was assayed spectrophotometrically after the agar was dissolved in boiling 5 M NaClO₄ (B. J. McCarthy and E. T. Bolton, J. Mol. Biol. 8:184, 1964).

The percentage of labeled DNA fragments (hereafter referred to by an asterisk: example, *nov**) bound by whole embedded DNA strands from homologous or heterologous bacteria was determined by the "tea bag" modification (B. J. McCarthy and B. H. Hoyer, Proc. Natl. Acad. Sci. U.S. 52:915, 1964) of the method of McCarthy and Bolton. The ratio of embedded to fragmented DNA was 200:1 in all experiments.

In hybridization experiments with *nov** or *tul**, a distinct reciprocal relationship was found between the DNA of these two species (Fig. 1A and 1B). Only a slight relationship was demonstrated between either of these species and the other three, whether *nov** and *tul** were used (Fig. 1A and 1B) or *mult** and *pes** (Fig. 1C and 1D). In all experiments, particularly those employing labeled fragments of *mult* (Fig. 1C), this species was found to be unlike the others.

Labeled DNA of *pes* was bound to nearly the same extent by both *pes* and *psTB*, indicating genetic similarity between these two species (Fig. 1D). DNA from *Ui* reacted to a significant degree with *pes* only.

These findings indicate that the *Pasteurella* group is made up of several subgroups whose members are more closely related to each other than to members of any other subgroup. Subgrouping within the genus *Pasteurella* has also been recognized by earlier investigators and has stimulated a proposed division of the genus, with *P. tularensis* and *P. novicida* being placed in a new genus *Francisella* (K. A. Dorofer, Symp. Res. Work Inst. Epidemiol. Microbiol. Chita 1:177, 1947; C. B. Philip and C. R. Owen, Intern.

FIG. 1. Extent of hybridization between ³²P-labeled DNA fragments and agar-embedded whole DNA from seven species of bacteria, including five from the genus *Pasteurella*.

Bull. Bacteriol. Nomencl. Taxon. 11:67, 1961) and *P. pestis* and *P. pseudotuberculosis* in another genus *Yersinia* (K. F. Meyer, in T. G. Hull, *Diseases Transmitted from Animals to Man*, Charles C Thomas, Publisher, Springfield, Ill.). The data reported here, based on a different

method of species comparison, add support to this proposal.

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