Lack of Relatedness of *Legionella pneumophila* to *Cytophagaceae*, "*Pasteurellaceae*," and *Kingella*

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Deoxyribonucleic acid from *Legionella pneumophila* showed little or no relatedness to deoxyribonucleic acids from members of the families *Cytophagaceae* and "*Pasteurellaceae*" (not on Approved Lists of Bacterial Names [Skerman et al., ed., Int. J. Syst. Bacteriol. 30:225-420, 1980]) or from *Kingella kingae*. These data support the recent recognition of *L. pneumophila* as a new species.

The Legionnaires disease bacterium was recently named *Legionella pneumophila* and placed in the new family *Legionellaceae* (2). The investigators stated that it was impossible to prove that *L. pneumophila* is different from all described species at the levels of species, genus, and family. They listed 15 genera that had guanine-plus-cytosine contents in their deoxyribonucleic acids (DNA) similar to that of *L. pneumophila* and for which possible relatedness to *L. pneumophila* had not been tested (2). In the present study we tested deoxyribonucleic acid (DNA) relatedness between *L. pneumophila* and species of *Cytophagaceae* and "*Pasteurellaceae*" (not on Approved Lists of Bacterial Names [5]) and *Kingella kingae*.

The strains used in this study are listed in Table 1. Detailed information on the *L. pneumophila* strains (2, 3), the strains of "*Pasteurellaceae*" (S. Pohl, Ph.D. thesis, Phillips-Universität, Marburg, Federal Republic of Germany, 1979), and the strains of *Cytophagaceae* (H. Behrens, Ph.D., thesis, Technischen Universität Carolo-Wilhelmina zu Braunschweig, Brunswick, Federal Republic of Germany, 1978) has been published. *K. kingae* CDC 5530, a human blood isolate from California, was received in the Special Bacteriology Section at the Centers for Disease Control in 1960. Methods for the cultivation of the bacteria, the preparation of both tritiated DNA and unlabeled DNA, and the hydroxyapatite assay for DNA relatedness have all been described (1-3; Behrens, Ph.D. thesis; Pohl, Ph.D. thesis).

DNA relatedness results obtained with tritiated DNA from *L. pneumophila* strain Philadelphia 1, the type strain, are shown in Table 1. *L. pneumophila* strains Albuquerque 1 and Pontiac 1 were 90% related to strain Philadelphia 1 in 60°C reactions. They were 77% related to Philadelphia 1 in 75°C reactions, in which only

closely related nucleotide sequences (less than 5% noncomplementary bases) can reassociate. All species of "*Pasteurellaceae*" and *Cytophagaceae* tested were unrelated to *L. pneumophila*.

Behrens (Ph.D. thesis) has recently studied DNA relatedness among *Cytophagaceae*, and Pohl (Ph.D. thesis) studied DNA relatedness among "*Pasteurellaceae*." The strains of *Cytophagaceae* and "*Pasteurellaceae*" used in the present study were chosen to represent the different hybridization groups demonstrated by the work of Behrens and of Pohl (Ph.D. theses). Collectively, these strains are 20% or more related to most, if not all, described species within these families. We are therefore confident that the lack of relatedness between *L. pneumophila* and the species tested provides a firm basis for concluding that *L. pneumophila* is not related to either *Cytophagaceae* or "*Pasteurellaceae*."

In 60°C reactions, *L. pneumophila* DNA showed 17% reassociation with DNA from *K. kingae*. At 75°C, these DNAs showed 2% reaction. This level of relatedness is reproducible and, although low, is higher than that observed between *L. pneumophila* and any other species tested here or previously (2, 3). Taken literally, 15 to 20% relatedness certainly indicates different species, and, at least genetically, different genera. This level of relatedness is, however, frequently observed in reactions between strains from different genera within the same family.

We tend to discount the significance of this low level of reassociation between *L. pneumophila* and *K. kingae* for the following reasons. *L. pneumophila* DNA was labeled in vitro. We have experienced occasional difficulties with nonspecific reassociation in in vitro-labeled DNA (D. J. Brenner, unpublished data). The thermal stability of double-stranded sequences formed between DNAs that are 15 to 25% related

TABLE 1. *Relatedness of DNA from L. pneumophila strain Philadelphia 1 to DNAs from K. kingae and strains of species in Cytophagaceae and "Pasteurellaceae"*

Bacterial source of unlabeled DNA ^a	% Relatedness with labeled <i>L. pneumophila</i> Philadelphia 1 DNA ^b	
	60°C	75°C
<i>Legionella pneumophila</i> Philadelphia 1	100	100
<i>L. pneumophila</i> Albuquerque 1	90	77
<i>L. pneumophila</i> Pontiac 1	89	77
<i>Kingella kingae</i> CDC 5530	17	2
<i>Pasteurella haemolytica</i> NCTC 9380	0	
" <i>P. piscicida</i> " ATCC 17911	0	
<i>P. multocida</i> NCTC 3195	1	
" <i>P. bettii</i> " NCTC 10535	1	
<i>Pasteurella</i> sp. NCTC 10699	0	
<i>Pasteurella</i> sp., BL group, NCTC 10547	2	
" <i>Actinobacillus seminis</i> " ATCC 15768	0	
<i>A. equuli</i> NCTC 3365	0	
<i>A. actinomycetemcomitans</i> NCTC 9710	1	
<i>Haemophilus influenzae</i> NCTC 8467	1	
<i>H. paragallinarum</i> NCTC 3438 ^c	1	
<i>H. parahaemolyticus</i> NCTC 8479	0	
<i>H. paraphrophilus</i> NCTC 10557	1	
<i>Cytophaga</i> sp. CYPE	0	
<i>Cytophaga</i> sp. CYH3	0	
<i>Cytophaga</i> sp. FK17	0	
<i>Cytophaga</i> sp. H72	0	
<i>Cytophaga</i> sp. H106	0	

^a CDC, Centers for Disease Control; NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection.

^b Actual reassociation in homologous *L. pneumophila* Philadelphia 1 reactions was between 45 and 80%. These were arbitrarily designated 100%, and heterologous values were normalized to them. A control reaction containing only labeled DNA was included in every experiment. The "label only" reaction was between 1 and 7%. Heterologous values were corrected for this background before normalization. All reactions were done at least twice.

^c "*H. paravium*" according to Hinz and Kunjara (4).

is almost always some 15 to 20°C lower than homologous DNA duplexes. Reassociated DNA formed between *L. pneumophila* and *K. kingae* is only 1 to 2°C less stable than homologous *L. pneumophila* DNA duplexes. *K. kingae* is culturally and biochemically very different from *L. pneumophila*. All of the following characteristics of *K. kingae* are the opposite of those for *L. pneumophila*: acid production from D-glucose and maltose, growth on blood agar, beta-hemolysis of blood agar, and fermentation in Hugh and Leifson's oxidation-fermentation test.

The results reported here support the recent classification of *L. pneumophila* as a new genus and species.

REPRINT REQUESTS

Address reprint requests to: Centers for Disease Control, Attention: D. J. Brenner, Enteric Section, 1-B311, Atlanta, GA 30333.

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